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91-05743/

Final Report

3.1-14

Occurrence and Significance of VA Mycorrhizal Symbiosis in Saskatchewan

Funded by ERDA and
The Agriculture Development Fund

ACKNOWLEDGEMENTS

The cooperation of Agriculture Canada research scientists and staff at research stations located at Indian Head, Swift Current, Scott and Melfort is gratefully acknowledged.

3.1-14

EXECUTIVE SUMMARY

Occurrence and Significance of VA Mycorrhizal Symbiosis in Saskatchewan Field Crops

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ABSTRACT

A study was undertaken to survey the distribution of vesicular-arbuscular mycorrhizae (VAM) in Saskatchewan soils and study factors which affect VAM populations and infectivity. High soil phosphorus levels and phosphorus fertilizer application reduced the level of VAM infection in spring wheat, lentil and field pea. VAM infection was similarly reduced in the year following summerfallow or canola. A survey of soils throughout Saskatchewan indicated the presence of at least eight different VAM species, some of which differ in their ability to promote growth. Thus VAM are ubiquitous in Saskatchewan and have a significant impact on the production of economically important crops.

SUMMARY

Introduction

The roots of most agriculturally important crops grown in Saskatchewan become infected with a fungi called vesicular-arbuscular mycorrhizae, or VAM. These fungi occur in most soils throughout the world and are, in fact, particularly beneficial to plant growth. When a VAM fungus infects plant roots, it extends thin fungal strands, called hyphae, into the surrounding soil. These fungal strands act as an extension of the root system and thus can improve crop growth by increasing the plants ability to obtain nutrients such as phosphorus, copper and zinc which might otherwise be unavailable to the plant. Other benefits of the VAM include improved drought resistance and salinity tolerance. This study was initiated to investigate the occurrence and importance of VAM in Saskatchewan soils and to determine factors which may affect VAM including cropping history and soil management practices.

Results

Field studies conducted during 1988 and 1989 indicate that VAM are present in sufficient numbers in Saskatchewan soils to be an important factor in crop growth. At study sites located throughout Saskatchewan, it was observed that crops such as spring wheat, lentil and field pea were heavily infected with VAM. This observation suggests that VAM plays an important role in the nutrition and growth of these economically important crops in Saskatchewan.

The dominate species of VAM present in Saskatchewan soils, and the relative abundance of these species, differed from location to location. Growth chamber studies have indicated that different VAM species differ in their ability to affect plant growth. These results suggest that the potential exists for the development of an effective VAM inoculant that could result in yield increases, particularly if applied to soils in which the indigenous VAM species is not an effective growth promoter. Isolation and identification of the different VAM species from Saskatchewan soils accomplished during the course of this research project represents an important step in the development of an effective VAM inoculant.

A number of factors were observed to play a role in the development of VAM infection in crop roots. For example, the extent of VAM infection was related to available soil phosphorus at seeding; generally high levels of available soil phosphorus resulted in a reduction in the levels of VAM infection in the crop roots. Similarly, application of fertilizer phosphorus subsequently reduced the levels of VAM infection. Thus, where phosphorus is limiting to crop growth, it is likely that VAM play a particularly important role in the procurement and uptake of this nutrient.

Crop rotations were also observed to be an important factor in VAM infection. Although most crops grown in Saskatchewan are subject to VAM infection, canola is resistant. It was observed that when this resistant crop was grown in a crop rotation, the level of VAM infection in the crop grown on the canola stubble was generally reduced. It is likely that during the canola year, VAM spores continued to germinate in the soil but were unable to find a suitable host crop to infect. Alternatively, other soil microorganisms may have attacked and destroyed VAM spores. As a consequence, the spores began to die off during the canola year, leaving fewer spores to infect the subsequent crop. The inclusion of summerfallow in a rotation generally had a similar effect on the levels of VAM infection, i.e., crops grown on summerfallow exhibited reduced levels of VAM infection as compared to crops grown in a continuous rotation. In light of the adverse effect that non-host crops such as canola, and summerfallow have on the population of VAM spores in the soil, VAM spore numbers could be strictly limited in rotations which included a year of summerfallow followed by canola, resulting in a reduction in the levels of VAM infection in the following crop. In a soil containing only marginal levels of available phosphorus, a reduction in the levels of VAM infection could result in yield limitations or, alternatively, a greater need for phosphorus fertilization. Further research is required to determine the extent to which reduced spore numbers may be affecting the crop yields and phosphorus requirements.

Herbicide application was also investigated as a factor which may influence the levels of VAM infection in crops. Although results suggest that herbicide application may influence VAM infection, it is not known whether the herbicides directly affected the VAM, or if herbicide application altered crop growth, indirectly affecting the VAM. Alternatively, herbicide application may have influenced VAM infection through alteration of the weed spectrum associated with the different herbicides. Although many weeds are readily infected by VAM, some are resistant; thus, the presence of non-host weeds may have altered the subsequent production of VAM spores.

Impact

This study has significantly enhanced our knowledge regarding the occurrence and distribution of VAM in Saskatchewan soils. Widespread occurrence and heavy infection of wheat, lentil and field pea suggests that VAM play an important role in the nutrition and growth of these economically important crops. VAM population and infectivity are subject

to the influence of a number of factors, including crop rotation. This indicates that there exists the potential to manipulate indigenous VAM through management practices in order to derive the greatest benefits to crop growth. Moreover, eight different VAM species have been isolated and identified which represents an important step towards the development of effective VAM inoculum.

Biological inoculants are important to Saskatchewan agriculture, as evidenced by the recent proliferation of local biotechnology companies including Philom Bios., Esso Biologicals and MicroBio Rhizogen. The mandate of these companies is to develop and exploit biological inoculants for agricultural use. Because of the ubiquitous nature of VAM and the important role that VAM plays in crop nutrition and growth, factors affecting this fungi should be identified. Biological interactions between VAM and biological inoculants may influence the success of the VAM association. Thus, there is a need to identify and consider biological interactions between indigenous VAM and potential biological inoculants, such as plant growth-promoting rhizobacteria (PGPR).

(B) Final Report

Final Report Submitted to the Agriculture Development Fund
October 22, 1990

Project Title:	Occurrence and Significance of VA Mycorrhizal Symbiosis in Saskatchewan Field Crops.
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1.0 Abstract

A study was undertaken to survey the distribution of vesicular-arbuscular mycorrhizae (VAM) in Saskatchewan soils and study factors which affect VAM populations and infectivity. Pattern of VAM infection was observed to be host specific. High soil phosphorus levels and phosphorus fertilizer application reduced the level of VAM infection in spring wheat, lentil and field pea. VAM infection was similarly reduced in the year following summerfallow or canola. Herbicide application may influence the level of VAM infection in wheat. A survey of soils throughout Saskatchewan indicated the presence of at least eight different VAM species, including *Acaulospora* sp., *Gigaspora margarita*, *Glomus caledonium*, *G. fasciculatum*, *G. Geosporum*, *G. mosseae*, *G. pallidum*, *G. versiforme*. Thus VAM are ubiquitous in Saskatchewan and have a significant impact on the production of economically important crops.

2.0 Background and Objectives

Most agriculturally important crops coexist with fungal root endophytes forming beneficial symbiotic associations called vesicular-arbuscular mycorrhizae (VAM). The Cruciferae and the Chenopodiaceae are the only families containing a number of crops of agricultural significance that do not form symbiotic associations with VAM fungi (see review by Gerdemann, 1968). Geographically, the association is ubiquitous, occurring in arctic, temperate and tropical regions (Powell and Bagyaraj, 1984).

Improved crop growth associated with VAM infection is often attributed to enhanced nutrient uptake (Abbott and Robson, 1984). Following initial infection, extramatrical hyphae extend several centimeters into the surrounding soil, effectively extending the soil volume explored and the absorptive surface area of the root system (see review by Mosse et al., 1981; Schenck 1982). As a result, the fungal hyphae are able to procure relatively immobile elements such as phosphorus (Hattingh et al., 1973; Rhodes and Gerdemann, 1975) and several micronutrients, including copper and zinc (Cooper and Tinker, 1978; Pacovsky, 1986), from beyond the zone of depletion at the root surface. Other benefits of mycorrhizal infection in some crops may include improved drought resistance (Safir et al, 1971) and salinity tolerance (Hirrel and Gerdemann, 1980), enhanced nodulation of legume roots by nitrogen fixing rhizobium and increased disease resistance (see review by Bagyaraj,1984).

Rising fertilizer costs have generated an interest in using VAM as a field crop

inoculant. Inoculation of some crops, even under field conditions, has been reported to improve growth and nutrition (Bagyaraj and Sreemula, 1982; Hayman et. al. 1975)). However, responses to VAM inoculation have been variable (Clarke and Mosse, 1981; Graw, 1979; Jenson, 1982, 1984) and reflect the large number of environmental and edaphic factors which may affect VAM infection including soil temperature, light intensity (Furlon and Fortin, 1973, 1977), pesticide application (Boatman et al., 1978; Manjunath and Bagyaraj, 1984), soil pH (Gilmore, 1971) and soil phosphorus (Hayman, 1980).

Kucey (1980) surveyed several central and east central Saskatchewan soils and concluded that mycorrhizal fungi are present in sufficient numbers in Saskatchewan soils to be an important factor in crop growth. Furthermore, he observed that virgin soils contained more spores than cultivated soils and concluded that management practices such as summerfallowing and crop rotation might affect mycorrhizal spore numbers. Similarly, Germida (1988) reported that herbicide application may reduce mycorrhizal infection of field pea and lentil grown under Saskatchewan conditions. Other studies have indicated that previous cropping history and fertilizer application can influence VAM fungi populations (Hayman, 1980); however, this research was conducted in England and may not be applicable to the western Canadian agroclimate.

In light of the important role that VAM play in plant nutrition, it is critical that factors which affect VAM numbers and infectivity, be identified. Having identified factors that affect VAM, management practices can be recommended which both minimize any harmful effects on mycorrhizal populations as well as encourage effective effective symbiotic relationships between indigenous VAM and susceptible field crops. In addition, identification and isolation of VAM from Saskatchewan soils represents an important initial step towards the development of a commercial VAM inoculant.

The objectives of this study were:

(1) to survey the distribution of VAM genera across the major Saskatchewan soil zones (Brown, Dark Brown, Black and Gray) and determine the infectivity of VAM in crops typically grown in each soil zone,

(2) to study factors which may affect mycorrhizae populations including cropping sequence, soil management practices, and herbicide application; and identify management practices which encourage effective symbiotic relationships between indigenous VAM and susceptible crops,

(3) to isolate, assess and develop mycorrhizal inoculants for use in Saskatchewan.

Note: Due to restricted funding of this project, studies regarding the development of an effective VAM inoculant were necessarily limited.

3.0 Experimental Methods

3.1 Field Studies

3.1.1 1988 Field Survey of Commercial Fields of Wheat, Lentil and Field Pea.

VAM infection in commercial fields of spring wheat, lentil and pea was monitored during the 1988 growing season at 12 study sites, representative of the major soil zones in Saskatchewan (Table 1). At three of the study sites, i.e., Aberdeen, Englefeld and Glenavon, samples were obtained from adjacent fields under different crop rotations

Table 1. Field sites monitored during the 1988 growing season.

Soil Zone	Experimental Location	Crop Rotation 1987	1988*
Brown	Outlook	wheat	wheat
		wheat	lentil
	Sceptre	fallow	wheat
		wheat	lentil
	Lucky Lake	fallow	wheat
	Kindersley	wheat	wheat
Dark Brown	Semans	wheat	wheat
		wheat	lentil
	Watrous	wheat	wheat
		wheat	lentil
	Aberdeen	wheat	wheat
Black	Englefeld	wheat	wheat
		wheat	lentil
	Leroy	wheat	wheat
		wheat	lentil
	Waldheim	wheat	field pea
	Glenavon	wheat	field pea
Gray	Stenen	wheat	wheat
		wheat	field pea

* - crops sampled during the 1988 field season

Plants were sampled throughout the growing season at times coinciding with various physiological crop stages . Twenty to 30 plant roots were randomly sampled at each study site (an area of 200 $m^{2)}$ by excavating the roots and surrounding soil to a depth of 15-20 cm. Bulk soil and root samples were then transported back to the laboratory for processing.

Composite root samples from each site were gently washed and divided into three subsamples. Samples were cleared and stained (Phillips and Hayman, 1970) and fifty randomly selected root segments (1 cm in length) from each subsample were examined for presence or absence of VAM hyphae (i.e., percent infection).

In addition, at each study site, 20 soil cores (2.5 cm diameter, 15 cm depth) were

collected from random locations and combined. Composite samples were then sieved (2 mm) and stored, field moist, at 8°C. Four 100 g subsamples were taken from each composite soil sample and the number of VAM spores was determined in each. Spores were recovered by wet sieving (250 and 106 µm sieve) and decanting (Gerdemann and Nicolson,1963), purified using a sucrose gradient technique (Mertz et al., 1979), tentatively identified (Schenek and Perez, 1988) and counted. Spore numbers were expressed on a dry soil basis (80°C, 24 h).

A modified MPN technique (Porter, 1979, Powell, 1980), consisting of a 10-fold dilution series (to 10^{-5}), was used to estimate the population of infective VAM propagules in soils.

3.1.2 1989 Field Survey of Commercial Fields of Wheat, Lentil and Barley.

VAM infection in commercial fields of spring wheat, lentil and barley was monitored during the 1989 growing season at eight study sites, representative of the four major soil zones in Saskatchewan (Table 2). At each site, adjacent fields cropped to either lentil, wheat, barley or canola, were sampled. It was assumed that the physical and chemical properties of the soils in these adjacent field were similar, and thus the number of VAM spores present in the spring of 1989 would adequately reflect the influence of the previous crops in the rotation.

Table 2. Field sites monitored during the 1988 growing season.

Soil Zone	Experimental Location	Crop Rotation 1987	1988	1989*
Brown	Outlook	wheat	wheat	lentil
		wheat	wheat	wheat
	Lucky Lake	fallow	wheat	lentil
		wheat	wheat	wheat
		wheat	fallow	wheat
Dark Brown	Semans	fallow	canola	barley
		wheat	wheat	barley
		wheat	barley	lentil
	Watrous	fallow	canola	wheat
		wheat	fallow	wheat
		fallow	wheat	wheat
Black	Englefeld	wheat	wheat	barley
		fallow	canola	barley
		fallow	canola	canola
	Glenavon	wheat	wheat	wheat
		wheat	canola	lentil
		wheat	canola	wheat
Gray	Stenen	wheat	canola	barley
		wheat	wheat	barley
		wheat	wheat	wheat
	Medstead	wheat	canola	barley
		wheat	fallow	barley
		wheat	wheat	barley

* - crops sampled during the 1989 field season

All study sites were located on undulating landforms; thus, to minimize the effects of spatial variability, care was taken to obtain root samples from the top, mid, and foot slope positions. Plants were sampled throughout the growing season at times coinciding with various physiological crop stages . Fifteen plant roots were sampled from each of the three slope positions within a 100 m^2 area by excavating the roots and surrounding soil to a depth of 15-20 cm. Bulk soil and root samples were then transported back to the laboratory for processing. Thirty soil cores were also collected from each site at seeding and at harvest and bulked. These soil samples were later used in the determination of spore numbers.

Root samples were gently washed to remove adhering soil and stained (Phillips and Hayman, 1970). Percent VAM infection was determined using the grid-line intersect method (Giovannetti and Mosse, 1980).

A modification of the method described by Mertz et al (1979) was used in the determination of spore numbers. Four 100 g subsamples were taken from each composite soil sample and the number of VAM spores were determined. Spores were recovered by wet sieving and decanting through a 106 µm and 250 µm sieve. The sievings contained a mixture of spores and organic material and spores were further purified from the organic material using a 30% sucrose and water gradient centrifugation technique. The spores that accumulated on the interface between the sucrose and the water were collected by aspiration. Spores were subsequently washed of excess sucrose and collected in 50 ml of water. Two 5 ml aliquots of this suspension were then uniformly spread on a gridded filter paper and spore numbers were counted under a stereomicroscope. Spore numbers are expressed on an oven dry soil basis (80° C, 24 hr).

VAM spores isolated from the study soils were permanently mounted using polyvinyl alcohol-lactic acid-glycerol (PVLG) mountant (Schenck and Yvonne, 1988). Standard taxanomic keys were then used to identify the VAM spores (Schenck and Perez, 1988).

3.1.3 1989 Field Study - The Effect of Long-term Crop Rotations and Fertilizer Application on VAM Infection of Spring Wheat.

The effect of crop rotation and long-term fertility regime on VAM spore population and infection of spring wheat was investigated during the 1989 field season. Soil and root samples were collected from four long-term rotation research experiments previously established by Agriculture Canada. These long-term rotation studies were located on Agriculture Canada Research Stations at Indian Head, Swift Current, Melfort and Scott. Characteristics of the study sites are given in Table 3.

Table 3. Characteristics of the Agriculture Canada long-term rotation study sites.

Agriculture Canada Research Station	Soil Classification	Soil Association	Year Rotation Study Initiated
Swift Current	Brown Chernozem	Wood Mountain loam (Mitchell et al., 1944)	1967
Indian Head	Dark Brown Chernozem	Indian Head clay (Clayton and Ellis, 1952)	1958
Scott	Orthic Dark Brown Chernozem	mixed Elstow and Shallow Elstow loam (Clayton and Ellis, 1952)	1965
Melfort	Orthic Deep Black Chernozem	Melfort silty clay (Clayton and Ellis, 1952)	1959

The long-term fertility regimes and rotations varied considerably from site to site, reflecting the differences in predominate cropping practices in each of the areas. Furthermore, not all of the existing rotation treatments at each of the sites were deemed to be relevant to this study and, as a result, only selected treatments were sampled at each site. All treatments were replicated four times with the exception of the Swift Current rotations where treatments were replicated three times. The rotation treatments sampled at each site are outlined in Table 4.

Table 4. Long term fertility treatments and rotations at Swift Current, Indian Head, Scott and Melfort.

Treatment[a] (rotation)	Swift Current	Indian Head	Scott	Melfort
	Fertilizer treatment (kg ha^{-1})			
cont. ***WH*** (fert.)	3.7 -20.8 N, 17.1 P_2O_5	83 N, 25 P_2O_5	46 N, 25 P_2O_5	58 N, 31 P_2O_5
cont. ***WH*** (unfert.)	NA[b]	0 N, 0 P_2O_5	NA	0 N, 0 P_2O_5
SF - ***WH*** (fert.)	3.7 N, 17.1, P_2O_5	5 N, 25 P_2O_5	11 N, 30 P_2O_5	24 N, 31 P_2O_5
SF - ***WH*** (unfert.)	NA	0 N, 0 P_2O_5	NA	NA
SF - ***WH*** - WH (fert.)	3.7 N, 17.1 P_2O_5	5 N, 24 P_2O_5	6.6 N, 31.6 P_2O_5	41 N, 31 P_2O_5
SF - ***WH*** -WH (unfert.)	0 N, 0 P_2O_5	0 N, 0 P_2O_5	NA	0 N, 0 P_2O_5
SF - WH - ***WH*** (fert.)	3.7-49.4 N, 17.1 P_2O_5	83 N, 25 P_2O_5	40 N, 31 P_2O_5	37 N, 31 P_2O_5
SF - WH - ***WH*** (unfert.)	0 N, 0 P_2O_5	0 N, 0 P_2O_5	NA	0 N, 0 P_2O_5
SF - CN - WH (fert.)	NA	NA	46 N, 25 P_2O_5	NA
SF - CN - ***WH*** -WH (fert.)	NA	NA	NA	57 N, 31 P_2O_5
SF - CN - WH - ***WH*** (fert.)	NA	NA	NA	57 N, 31 P_2O_5

a - SF denotes summer fallow, WH denotes spring wheat, CN denotes canola. The bolded and italicized letters denote the treatment and year of the rotation.
b - denotes "Not Available".

At each site, treatments were sampled at four times during the growing season. Sampling dates and corresponding days after planting are reported in Table 5. At each sampling date, soil and root samples were excavated to a depth of 15 cm, bagged, and transported to the laboratory. Roots were retrieved from the bulk samples by washing the soil with a gentle stream of water. Root samples were then stained (Phillips and Hayman, 1970) and placed in a glass petri dish marked with a one cm grid. Percent infection was determined microscopically by observing the presence or absence of VAM hyphae, arbuscules and vesicles at 100 random intersects.

Table 5. Sampling dates and corresponding days after planting (DAP) at Indian Head, Swift Current, Scott and Melfort.

	Experimental Location							
Sampling	Indian Head		Swift Current		Scott		Melfort	
Period	Date	DAP	Date	DAP	Date	DAP	Date	DAP
1	18/05	18	31/05	25	1/06	27	16/06	27
2	9/06	40	20/06	45	21/06	47	6/07	47
3	29/06	60	10/07	65	11/07	67	26/07	67
4	19/07	80	28/07	85	29/07	89	15/08	88

Bulk soil samples were also collected from each treatment at the first sampling date for determination of spore numbers. Spores were isolated from the soil using a modified sucrose centrifugation technique (Jenkinson, 1964). The modification consisted of vigorously stirring 50 g of soil in 1000 ml of tap water for three minutes. The mixture was then allowed to stand for 30 seconds after which it was decanted through a 60 mesh screen and the supernatant was collected. The residue was resuspended in the same manner and again the supernatant was decanted through a 60 mesh screen and collected. The supernatant was then passed through a 140 mesh screen and the material retained on the screen was transferred to a 50 ml centrifuge tube. The material was centrifuged at 1800 rpm for 5 minutes and the supernatant was then decanted. A 30 percent sucrose solution was added to the centrifuge tube and the pellet was resuspended. Again the tube was centrifuged at 1800 rpm for two minutes. The supernatant was decanted and all of the supernatant was decanted through a 140 mesh screen. The retained spores were washed with water and transferred to a beaker containing 100 ml of water. Duplicate 10 ml aliquots of this solution were obtained and the spores contained in each aliquot were collected on separate 0.8 um millipore filter membranes. The filters were then examined microscopically (10x) and the number of spores were recorded.

Soil tests for available nitrogen and phosphorus were conducted by the individual Agriculture Canada research stations. Soil tests were conducted prior to seeding at all but the Indian Head experimental site where soil tests were conducted in the fall of the previous year. Soil test results for nitrogen and phosphorus were obtained through the cooperation of Agriculture Canada and are reported in Table 6.

Table 6. Levels of available soil nitrogen and phosphorus at Swift Current, Indian Head, Scott and Melfort, 1989 Field Study.

Treatment[a] (rotation)	Swift Current		Indian Head		Scott		Melfort	
	Nutrient content (ug g-1)							
	NO3-N[b]	P[c]	NO3-N	P	NO3-N	P	NO3-N	P
cont. ***WH*** (fert.)	NA[d]	NA	21.5	17.0	33.5	12.8	26.5	7.2
cont. ***WH*** (unfert.)	NA	NA	NA	NA	NA	NA	6.6	5.6
SF - ***WH*** (fert.)	67.2	30.8	56.5	24.5	80.0	7.8	36.2	13.4
SF - ***WH*** (unfert.)	NA	NA	NA	NA	NA	NA	NA	NA
SF - ***WH*** - WH (fert.)	77.6	26.0	34.0	18.3	77.5	7.2	19.2	8.1
SF - WH -***WH*** (fert.)	65.1	38.7	10.8	21.4	39.0	7.2	36.0	13.4
SF - ***WH*** - WH (unfert.)	87.8	14.7	NA	NA	NA	NA	29.1	6.0
SF - WH - ***WH*** (unfert.)	65.9	24.3	NA	NA	NA	NA	20.0	8.4
SF - CN - WH (fert.)	NA	NA	NA	NA	37.5	8.8	30.3	19.1
SF - CN - ***WH*** -WH (fert.)	NA	NA	NA	NA	NA	NA	68.4	20.1
SF - CN - WH - ***WH*** (fert.)	NA	NA	NA	NA	NA	NA	31.5	16.2

a - SF denotes summer fallow, WH denotes spring wheat, CN denotes canola. The bolded and italicized letters denote the treatment and year of the rotation.
b - available NO3-N to 60 cm.
c - available P to 15 cm.
d - denotes "Not Available".

3.1.4 1989 Field Study - The Effect of Herbicide Application on VAM Infection of Spring Wheat.

The effect of herbicide application on VAM spore population and infection of spring wheat was investigated during the 1989 field season. Soil and root samples were collected from a long-term research experiments previously established by Agriculture Canada. This long-term study was located at Loon Lake at an off-station of the Agriculture Canada Research Station at Scott, Saskatchewan. The site at Loon Lake was located on a Grey-Wooded soil.

Initiated in 1982, the experiment itself was originally designed to determine the effect of cropping practices on weed populations in a continuous wheat rotation. Thus treatments consisted of varying tillage practices, stubble heights, fertility levels and herbicide treatments. Not all of the treatments in the experiment were deemed relevant to this study and, as a result, only selected treatments were sampled. All treatments were replicated four times. Treatments sampled are outlined in Table 7.

Table 7. Description of herbicide treatments sampled at Loon Lake, 1989.

Treatment	Herbicide Application	Fertilizer Application
1	untreated	none
2	Hoegrass (0.70 kg ha-1) and Glean (0.02 kg ha-1), separate application	none
3	Hoegrass (0.70 kg ha-1)/ Torch (0.35 kg ha-1) tank mix	none
4	Hoegrass (0.70 kg ha-1) and Buctril M (0.56 kg ha-1), separate application	none
5	untreated	80 lb N/acre broadcast 40 lb P_2O_5/ acre seed placed
6	Hoegrass (0.70 kg ha-1) and Glean (0.02 kg ha-1), separate application	80 lb N/acre broadcast 40 lb P_2O_5/ acre seed placed
7	Hoegrass (0.70 kg ha-1)/ Torch (0.35 kg ha-1) tank mix	80 lb N/acre broadcast 40 lb P_2O_5/ acre seed placed
8	Hoegrass (0.70 kg ha-1) and Buctril M (0.56 kg ha-1), separate application	80 lb N/acre broadcast 40 lb P_2O_5/ acre seed placed

Treatments were sampled at four times during the growing season; 20, 40, 60 and 81 days after planting. At each sampling date, soil and root samples were excavated to a depth of 15 cm, bagged, and transported to the laboratory. Roots were retrieved from the bulk samples by washing the soil with a gentle stream of water. Root samples were then stained (Phillips and Hayman, 1970) and placed in a glass petri dish marked with a one cm grid. Percent infection was determined microscopically by observing the presence or absence of VAM hyphae, arbuscules and vesicles at 100 random intersects.

Bulk soil samples were also collected from each treatment at the first sampling date

for determination of spore numbers. Spores were isolated from the soil using a modified sucrose centrifugation technique (Jenkinson, 1964) as previously described (see Section 3.1.3). Soil tests are not available for this experimental location.

Although this experiment was originally designed as a split, split plot experiment, only selected treatments were sampled. As a result, the experiment was statistically analyzed as a split - plot experiment with herbicide combinations as the main plot and fertility levels as the subplot.

3.2 Growth Chamber Studies

3.2.1 Growth Chamber Experiment #1 - The Development of VAM Infection in Wheat, Lentil and Field Pea.

Growth chamber studies were initiated to investigate VAM infection pattern of wheat, lentil and field pea roots under controlled conditions. The experiments were conducted using a completely randomized design consisting of five replicates. Wheat, lentil and field pea were planted in pots containing 1 kg of unammended soil. Following germination and emergence, pots were thinned to contain two plants per pot. Soil was maintained at field capacity through daily additions of sterile, distilled, deionized water.

The soil used in this experiment was an Elstow loam which is a Dark Brown Chernozem according to the Canadian system of soil classification. The soil was collected from the Ap horizon (surface 0-15 cm) in the fall of 1988. The soil was air-dried and sieved to pass a 2 mm sieve prior to storage. The soil was stored in an air-dry condition. Immediately prior to use of the soil in the growth chamber experiment, various chemical and physical characteristics of the soil were determined (Table 8).

Table 8. Some chemical and physical characteristics of the soil used in Growth Chamber Experiment #1.

Soil Classification	Dark Brown Chernozem
Soil Association	Elstow loam
texture	loam
field capacity	24.6%
pH	7.2
conductivity (ms cm-1)	0.5
NO3-N (ug g^{-1})	150
P (ug g^{-1})	36.5
K (ug g^{-1})	290
SO4-S (ug g^{-1})	4.0
Cu (ug g^{-1})	1.3
Fe (ug g^{-1})	6.7
Zn (ug g^{-1})	2.4
Mn (ug g^{-1})	9.6

Plants were harvested at 20, 30, 40, 50 and 60 days after seeding. At each sampling date, both shoots and roots were retrieved. Roots were carefully washed by hand to remove all adhering soil. Root samples were cut into segments measuring approximately 1 cm and root length was determined using the grid-line intersect method (Newmann

1966). Root samples were then stained (Phillips and Hayman, 1970) and placed in a glass petri dish marked with a one cm grid. Percent infection was determined microscopically by observing the presence or absence of VAM hyphae, arbuscules and vesicles at 100 random intersects.

3.2.2 Growth Chamber Experiment # 2 - The Effect of Phosphorus Application on the Development of VAM Infection in Wheat.

A growth chamber experiment was initiated to investigate the effects of phosphorus application on VAM infection of spring wheat. The growth chamber experiment was conducted using a completely randomized design consisting of three phosphorus treatments (0, 15 and 75 µg g-1 P) and five replicates. A total of 25 pots were prepared for each treatment to accommodate 5 separate sampling periods.

The soil used in this experiment was an Elstow loam which is a Dark Brown Chernozem according to the Canadian system of soil classification. The soil was collected from the Ap horizon (surface 0-15 cm) in the fall of 1988. The soil was air-dried and sieved to pass a 2 mm sieve prior to storage. The soil was stored in an air-dry condition. Immediately prior to use of the soil in the growth chamber experiment, various chemical and physical characteristics of the soil were determined (Table 9).

Table 9. Some chemical and physical characteristics of the soil used in Growth Chamber Experiment #2.

Soil Classification	Dark Brown Chernozem
Soil Association	Elstow loam
texture	loam
field capacity	24.6%
pH	7.2
conductivity (ms cm-1)	0.5
NO3-N (µg g^{-1})	150
P (µg g^{-1})	36.5
K (µg g^{-1})	290
SO4-S (µg g^{-1})	4.0
Cu (µg g^{-1})	1.3
Fe (µg g^{-1})	6.7
Zn (µg g^{-1})	2.4
Mn (µg g^{-1})	9.6

Prior to seeding, 1000 g of air dry soil was placed into a polyethylene bag. Twenty mls of a nutrient solution was applied to each treatment at the following rates: 75 µg g^{-1} N as NH_4NO_3; 200 µg g^{-1} K as K_2SO_4 (109 µg.g^{-1}) and KCl (91 µg g^{-1}); and 50 µg g^{-1} S as $Mn.SO_4$ (3 µg g^{-1}), $ZnSO_4.7H_2O$ (2 µg g^{-1}), $CuSO_4.5H_2O$ (0.3 µg g^{-1}) and K_2SO_4 (45 µg g^{-1}). Nutrients were dissolved in distilled deionized water, pipetted onto the surface of the soil in each bag and the bags were then thoroughly shaken to ensure incorporation and even distribution of the nutrients within the soil. A second solution containing micronutrients was applied in the same manner at the following rates:0.6 µg g^{-1} Mo as $NaMoO_4.2H_2O$; 5 µg g^{-1} Mn as $MnSO_4.H_2O$; 4 µg g^{-1} Zn as $ZnSO_4.7H_2O$; 1.5 µg g^{-1} B as H_3BO_3; and 0.6 µg g^{-1} Cu as $CuSO_4.5H_2O$. Twenty and 100 mls of a third nutrient

solution, containing P as $CaH_4(PO_4)_2.H_2O$, was applied to soils receiving phosphorus treatments at application rates of 15 and 75 (µg g^{-1}), respectively. Following addition and incorporation of the nutrient solutions, distilled deionized water was added to the soil in each polyethylene bag to bring the soil to approximately 1/2 field capacity and the soils were again thoroughly shaken. The amended soils were then placed in 20 cm open drainage pots, covered with brown paper and allowed to incubate at 20 ° C for 24 hours.

Six spring wheat seeds were seeded at a depth of approximately 2 cm in each of the 75 pots. Following germination and emergence, each pot was thinned to contain two plants per pot. Following thinning, soils were maintained at field capacity through daily additions of distilled water.

Plants were harvested at 20, 30, 40, 50 and 60 days after seeding. At each sampling date, both shoots and roots were retrieved. Roots were carefully washed by hand to remove all adhering soil. Root samples were cut into segments measuring approximately 1 cm and root length was determined using the grid-line intersect method (Newmann 1966). Root samples were then stained (Phillips and Hayman, 1970) and placed in a glass petri dish marked with a one cm grid. Percent infection was determined microscopically by observing the presence or absence of VAM hyphae, arbuscules and vesicles at 100 random intersects.

3.2.3 Growth Chamber Experiment #3 - The Effect of Crop Rotation on the Development of VAM Infection in Wheat.

The effect of crop rotation on the development of VAM infection was investigated in a growth chamber experiment. Soils used in this experiment were collected from adjacent fields, located near Englefeld, which had been subjected to different crop rotations during the previous two growing seasons, i.e., canola - canola (C-C), canola - barley (C-B), and wheat - barley (W-B). The soils were collected in the fall of 1989.

In addition to investigating the influence of crop rotation on VAM infection, a second variable, namely soil dilution, was imposed on this experiment. Previous field studies had suggested that some crop rotations may adversely effect VAM infection simply by reducing the number of spores present in the soil. Thus, in order to determine whether the reduction in VAM infection following certain rotation was merely a reflection of reduced spore numbers or if some other factor may be responsible, in part, for the subsequent reductions in VAM infection, soil dilutions were included in this experiment. Soils from each rotation were diluted with coarse sand at soil:sand ratios of 0.5:1.5, 1:1, and 2:0, effectively reducing the spore numbers in the diluted soils. In order to compensate for the concomitant reduction in nutrient status of the diluted soils, treatments received a basal nutrient application.

The experiment was conducted using a split plot design, with soil dilutions as the mainplot and crop rotation as the subplot. All possible combinations of crop rotations and soil dilutions were employed, with a total of nine treatments, replicated four times. In addition, two replicates of the 0.5:1.5 and 1:1 soil:sand dilutions which did not receive any additional nutrient solution were included to ascertain whether nutrient additions were compensating fully for the effect of the sand dilution and whether the nutrient additions adequately maintained wheat growth.

Soil treatments were placed in 15 cm diameter plastic pots and seeded with five spring wheat seeds. Following germination and emergence, pots were thinned to two

plants per pot. Soil was maintained at 60% moisture holding capacity through daily additions of distilled water. At the termination of the experiment, both shoot and roots were retrieved. Roots were gently washed to remove any adhering soil. Root samples were then stained (Phillips and Hayman, 1970) and placed in a glass petri dish marked with a one cm grid. Percent infection was determined microscopically by observing the presence or absence of VAM hyphae, arbuscules and vesicles at 100 random intersects. Shoots were oven dried and oven dry weight recorded.

4.0 Results and Discussion

4.1 Field Studies

4.1.1 1988 Field Survey of Commercial Fields of Wheat, Lentil and Field Pea.

Factors influencing percent VAM infection, including crop rotation, available soil P and available soil moisture, were investigated at each of the twelve sites established during the 1988 growing season. Data suggest that percent VAM infection of spring wheat was influenced by available soil moisture at seeding (Table 10). In the Brown soil zone, infection levels were highest at the irrigated Outlook site. In contrast, VAM infection levels were lowest at Sceptre, where available spring soil moisture levels were low and drought conditions persisted throughout the growing season. Similarly, higher levels of VAM infection of lentil roots at Outlook as compared to relatively low levels observed at Sceptre can be attributed to differences in available spring soil moisture.

Table 10. Influence of available spring moisture and available soil phosphorous on the percent VAM infection of spring wheat, lentil and field pea grown during the 1988 field season.

Soil Zone	Location	Crop Rotation 1987	Crop Rotation 1988	Infection (%) Days after emergence 15-30	45-60	75-90	Available P (kg/ha)	Soil Moisture (cm)
Brown	Outlook	Wheat	Wheat	20	50	31	52	Irrigated
		Wheat	Lentil	94	96	100	22	Irrigated
	Sceptre	Fallow	Wheat	7	9		58	1
		Wheat	Lentil	11	49		24	1
	Lucky Lake	Fallow	Wheat	12	22	12	60	16
	Kindersley	Wheat	Wheat	14	20	13	47	10
D. Brown	Semans	Wheat	Wheat	36	41	27	108	7
		Wheat	Lentil	56	70	71	80	2
	Watrous	Wheat	Wheat	42	46	34	94	18
		Wheat	Lentil	58	81	87	77	8
	Aberdeen	Wheat	Wheat	40	44	26	90	10
Black	Englefeld	Wheat	Wheat	17	8	2	134	13
		Wheat	Lentil	52	59	79	64	13
	Leroy	Wheat	Wheat	8	3	2	128	8
		Wheat	Lentil	55	64	61	128	8
	Waldheim	Wheat	Pea	66	80	78	74	10
	Glenavon	Wheat	Pea	44	62	71	100	8
Gray	Stenen	Wheat	Wheat	18	32	14	54	20
		Wheat	Pea	62	88	97	24	20

Available soil P was also observed to influence the levels of VAM infection in spring wheat. Infection of wheat grown in the Black soil zone was relatively low as compared to wheat grown in the Dark Brown soil zone despite similar levels of available soil moisture. The two sites in the Black soil zone had been continuously fertilized with phosphate for the previous ten years and it is likely that high levels of residual P resulted in

a reduction in the extent of mycorrhizal infection.

Infection of wheat roots by VAM was most rapid during early growth stages (Fig. 1). Maximum infection of wheat roots generally occurred within 45-60 days of emergence, after which levels of infection declined. Infection of both lentil and field pea also occurred at a rapid rate during early growth stages, but reached a plateau within 45-60 days of emergence (Fig.2). Patterns of infection were similar in all four soil zones surveyed. At sites where more than one crop was sampled, wheat roots had a lower level of VAM infection than either lentil or field pea (Fig.2 and Fig.3). Moreover, microscopic observations indicated that arbuscules and vesicles were more prevalent in lentil and field pea roots than in wheat roots.

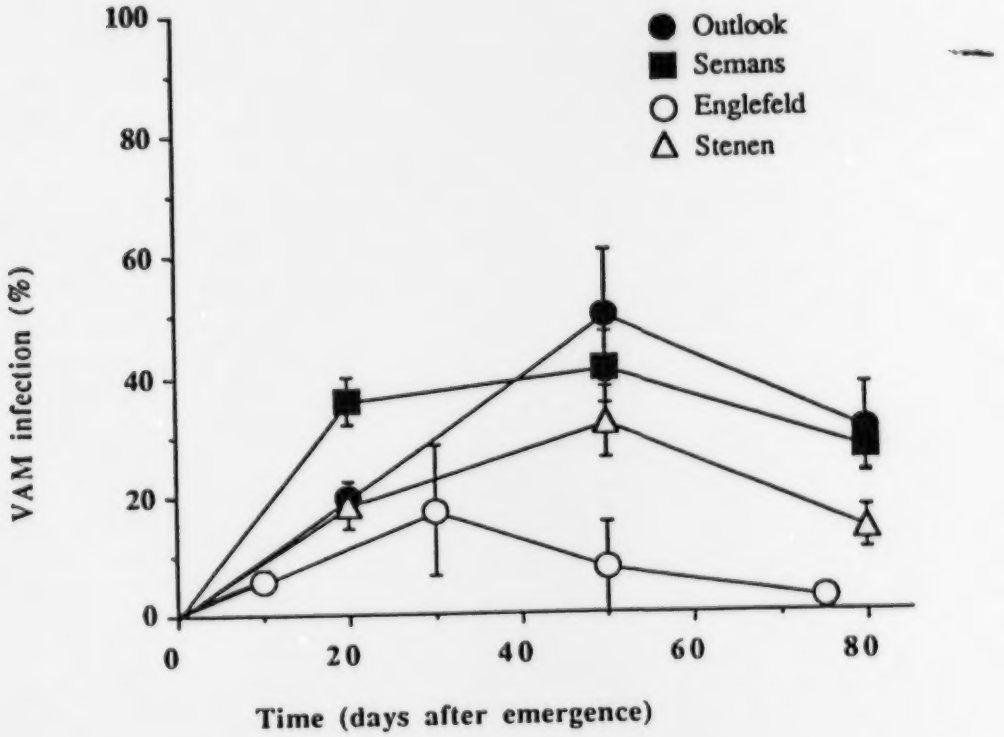

Figure 1. Extent and pattern of VAM infection in wheat roots in the four soil zones.

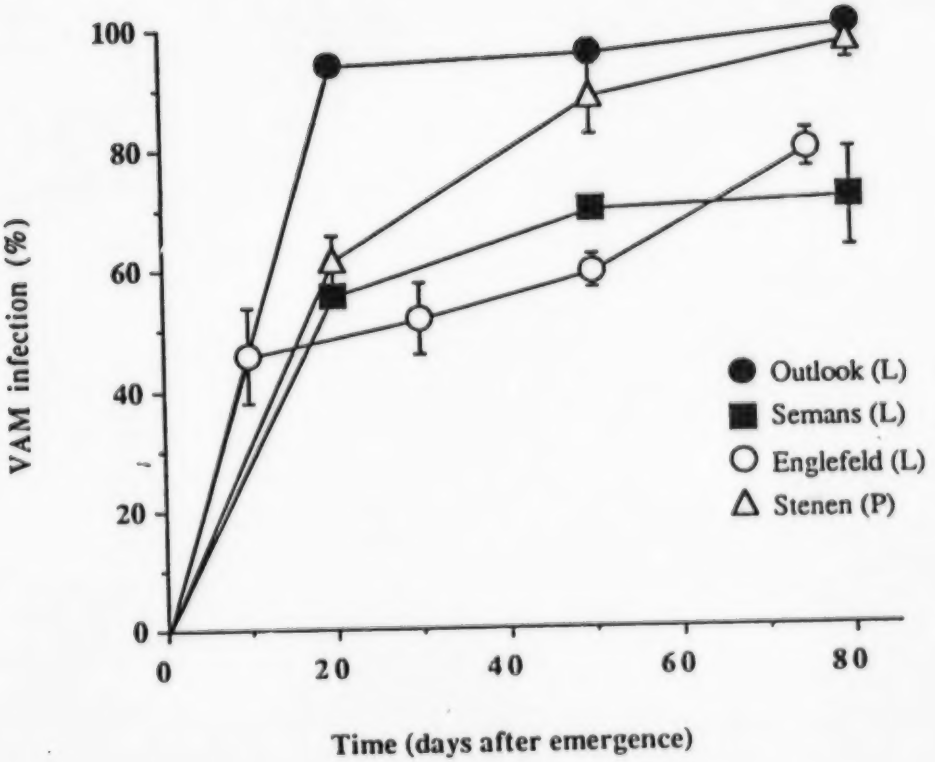

Figure 2. Extent and pattern of VAM infection in lentil (L) and field pea (P) roots in the four soil zones.

The effect of crop rotation was monitored at Aberdeen, Englefeld and Glenavon (Fig. 3). Adjacent fields in which different crop rotations had been grown were sampled throughout the growing season. At Aberdeen, levels of VAM infection were highest when wheat was grown in a continuous wheat rotation (Fig. 3a). It is likely that the continuous presence of spring wheat helped maintain high levels of percent infection by providing a constant inoculum source for subsequent crops. Summerfallow reduced infection of spring wheat in the following year. Infection of spring wheat was limited to the greatest degree in the rotation which included canola and summerfallow. Similar trends were observed at Englefeld (Fig. 3b) and Glenavon (Fig. 3c). The reduction in percent infection due to inclusion of canola in the crop rotation was much greater at Glenavon than at the other locations. When canola was grown with field pea, percent infection was unaffected by the presence of canola, whereas summerfallowing had a negative effect on infection.

At seeding , the number of VAM spores in soils ranged from 78-346 per 100 g soil (Table 11). The data indicate that fewer VAM spores are present in soils of the Brown soil zone than in soils of the Dark Brown, Black and Gray soil zones. There was no relationship between spore numbers and subsequent infection of crop roots

Table 11. VAM spore numbers, infective propagules and infectivity at the field study sites.

Soil Zone	Site	Spore numbers at seeding	Infection (%)	Infective propagules (MPN)
Brown	Outlook	118	94	220
	Sceptre	128	11	350
	Lucky Lake	78	ND*	220
	Kindersley	121	ND	79
Dark Brown	Semans	272	56	280
	Watrous	256	58	240
	Aberdeen	346	ND	490
Black	Englefeld	150	52	ND
	Leroy	125	55	280
	Waldheim	248	66	1400
Gray	Glenavon	262	44	180
	Stenen	246	62	140

* ND - not determined.

Microscopic investigation indicated that spores from more than one species of VA mycorrhizae were present in the soils sampled. The predominant VAM species were *G. mosseae, G. fasciculatum, G.pallidum, G. caledonium* and *Acaulospora spp.*.

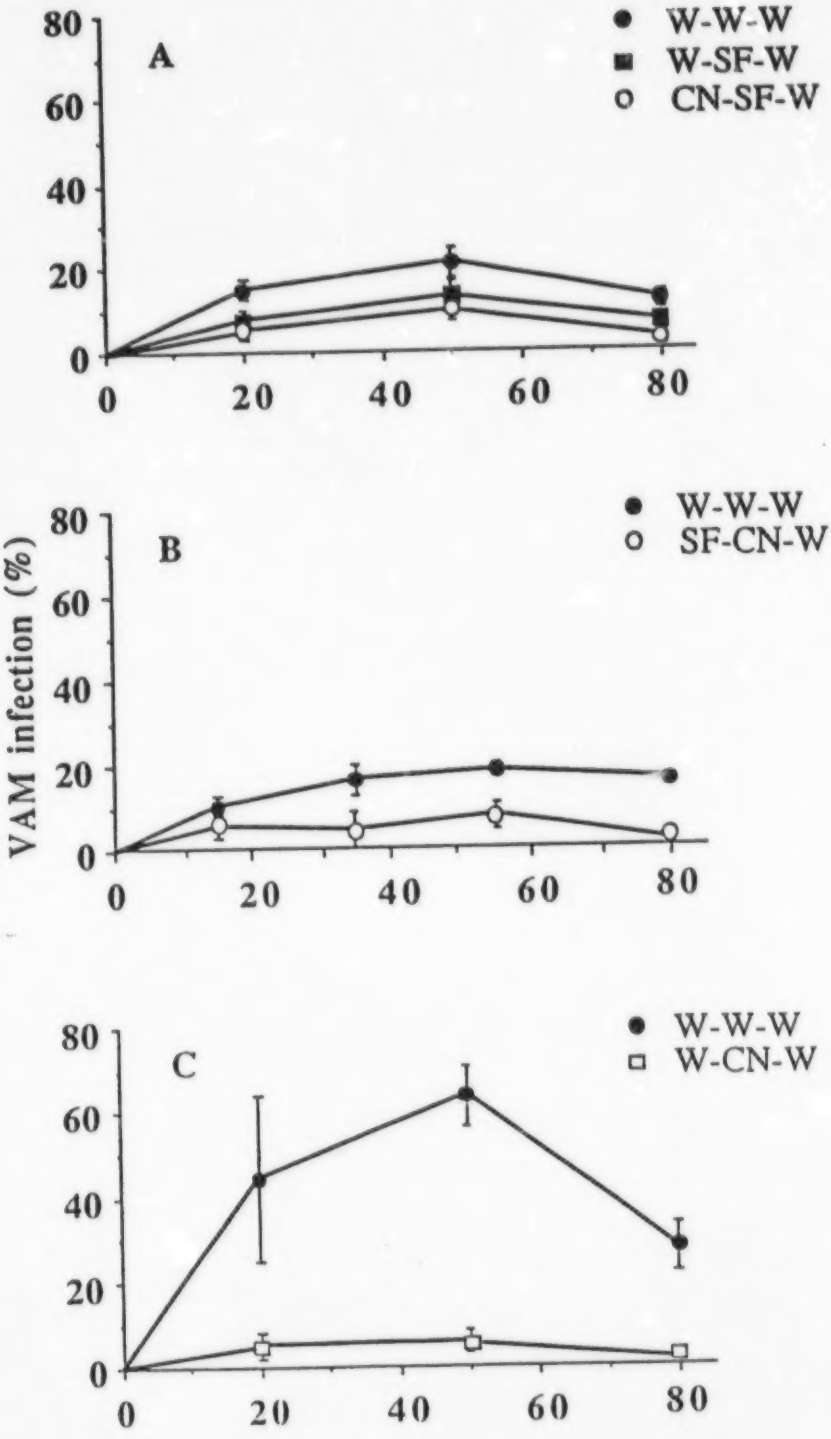

Figure 3. Effect of crop rotation, which includes spring wheat (SW), canola (C) and summerfallow (SF), on VAM infection of spring wheat grown in the Dark Brown (A) and Black (B and C) soil zones.

3.1.2 1989 Field Survey of Commercial Fields of Wheat, Lentil and Barley

The extent of VAM infection in wheat and lentil at the 1989 field sites is presented in Figure 4. The level of VAM infection of wheat ranged from 29-48% within forty days of emergence. Among the various study sites, VAM infection in wheat was highest in the Gray soil zone (Stenen) throughout the growing season . In the case of lentil, percent VAM infection ranged from 56-94% within 40 days of emergence. The two crops were grown in adjacent fields at only one site, i.e., Outlook and the percent VAM infection of lentil at this site was much higher than wheat. Because the lentil and wheat plots at other study sites were not adjacent, it was not possible to make direct comparison of their infection level. However, lentil maintained higher percent VAM infection than wheat irrespective of the study site. In the case of lentil, percent VAM infection increased continuously until 60-70 days after emergence whereas wheat infection increased continuously at only two sites (Lucky Lake and Stenen). At Watrous and Outlook, percent VAM infection of wheat decreased 40 days after emergence. This observation is in accordance with observations made in 1988 where it was observed that percent VAM infection of wheat decreased 45 days after emergence at all study sites. The apparent decline in infection likely resulted from a greater increase in root growth than in the development of the VAM infection. Alternatively, older, infected roots may have been sloughed off and replaced by newer, uninfected roots.

The relationship between available soil P and VAM infection of lentil and wheat was investigated in 1989, and is illustrated in Figures 5 and 6, respectively. The correlation coefficients between available P and percent VAM infection of lentil was 0.266 at the first sampling time, 0.481 at the second sampling time and 0.574 at the third sampling time. (Fig. 5). For wheat, these values were 0.134, 0.422 and 0.361, respectively (Figure 6). In the case of lentil, the relationships were statistically significant at all of the sampling times whereas in the case of wheat, the relationship was statistically significant only at the second and third sampling time. These relationships suggest that the observed variation in the extent of VAM infection was associated with differences in available soil P at these study sites.

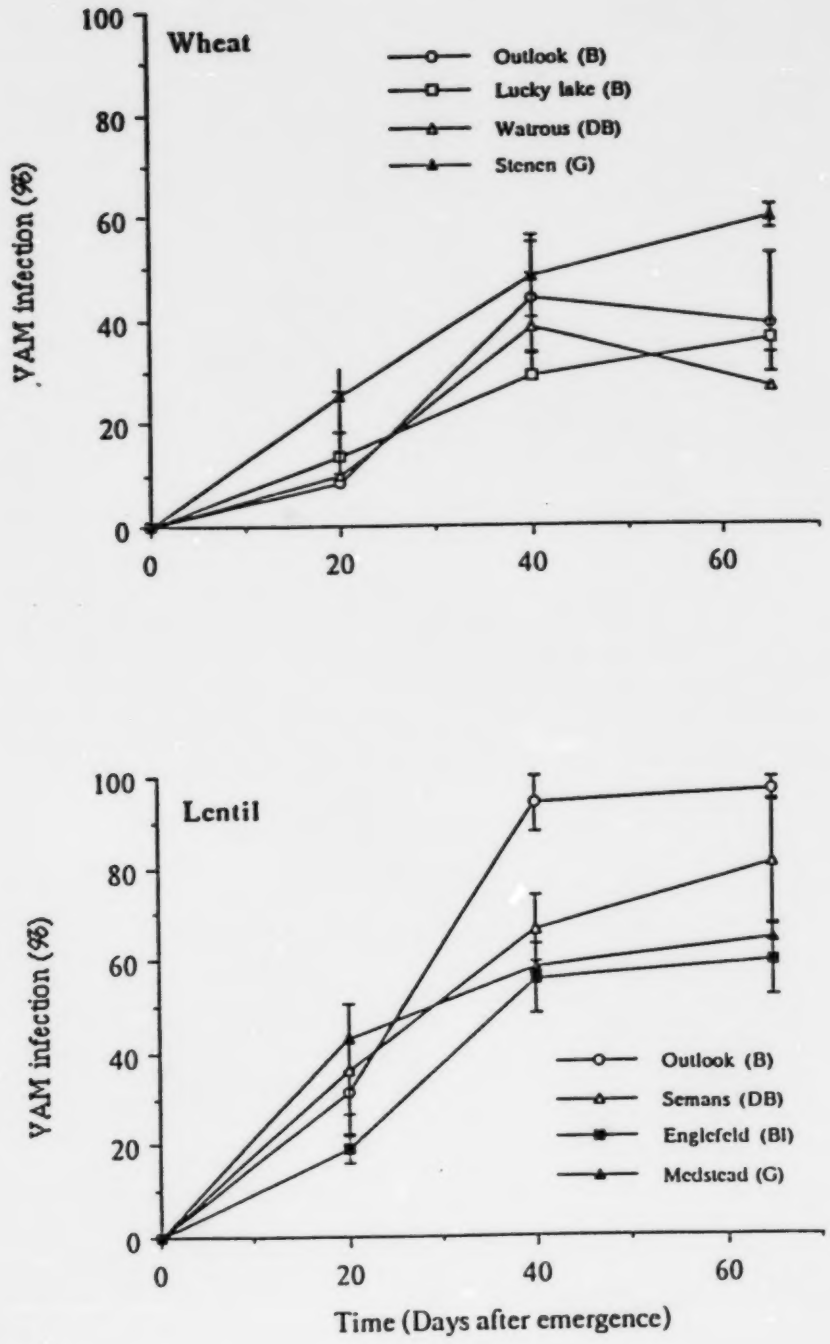

Figure 4. VAM infection of wheat and lentil grown in the Brown (B), Dark Brown (DB), Black and Gray (G) soil zone of Saskatchewan.

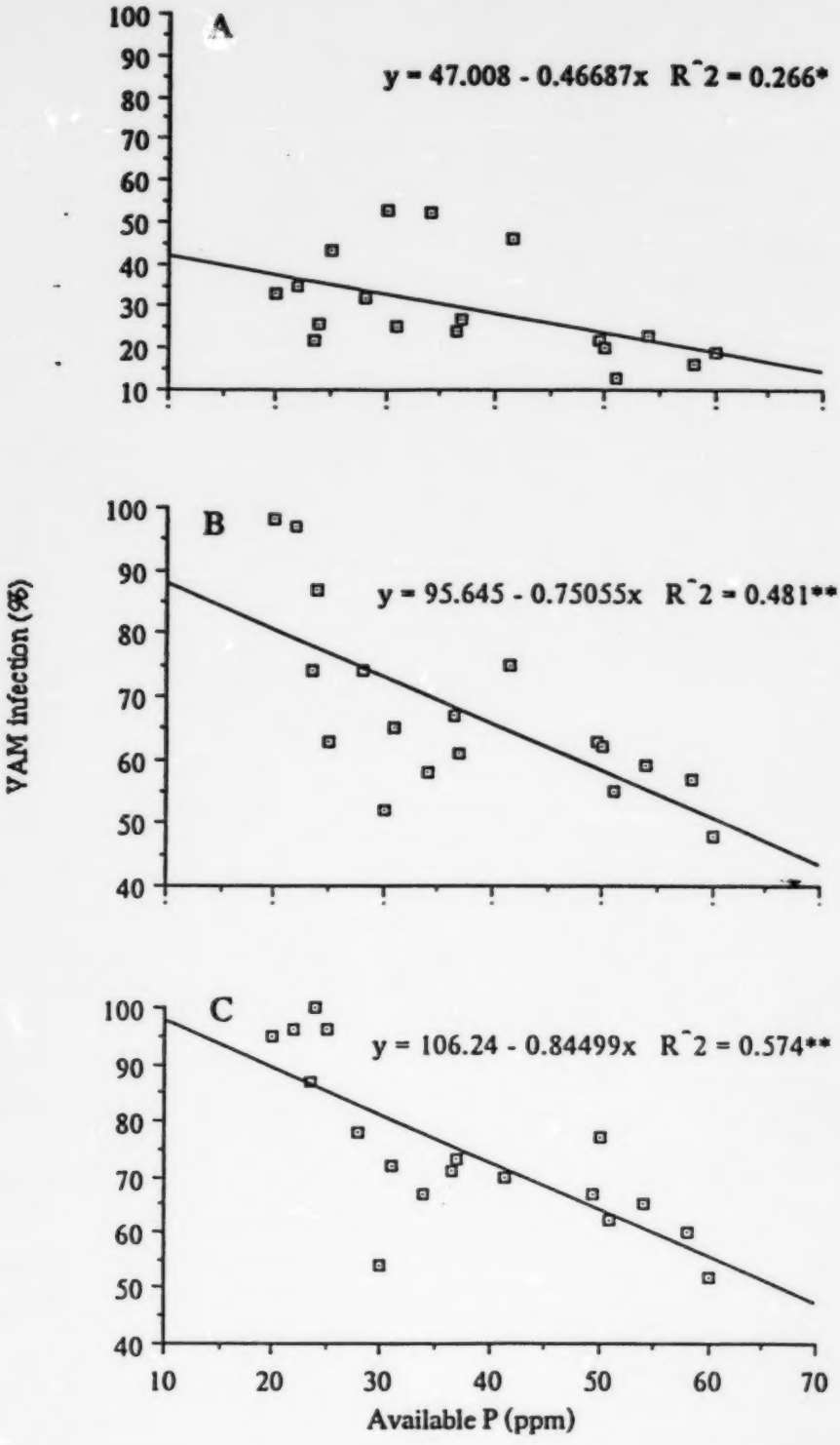

Figure 5. Relationship between available phosphorus and % VAM infection of lentil at 20 (A), 40 (B), and 65 (C) days after emergence at different study sites.

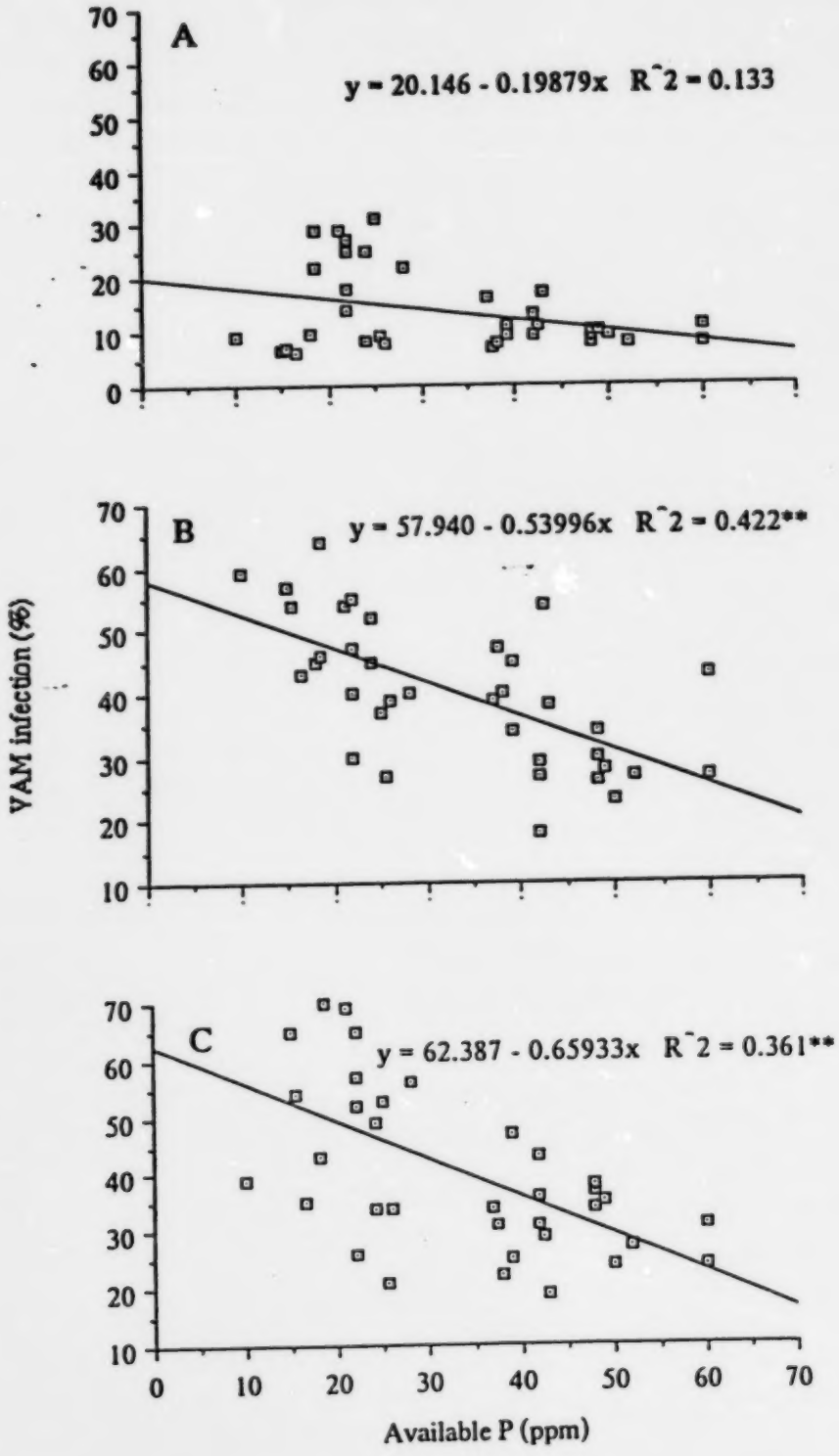

Figure 6. Relationship between available phosphorus and % VAM infection of wheat at 20 (A), 40 (B), and 65 (C) days after emergence at different study sites.

The effect of crop rotation on VAM spore populations is presented in Table 12. Spore numbers at seeding in 1989 were lower in all study sites where the previous crop (i.e., 1988) had been canola or if the field had been summerfallowed. The reductions were more evident in canola fields than in summerfallow fields at sites where adjacent canola and summerfallow fields had been sampled.

Table 12. VAM spore populations in as determined in the spring of 1989 in fields cropped to canola, wheat. or fallowed in 1988.

Site	Spore number				
	Canola	Summerfallow	Wheat	difference	*t* value
	Per 100 g soil[a]				
Lucky lake	-	1535	1787	252**	-5.248
Watrous	950	1085	-	135*	-3.366
	950	-	1227	277*	-2.837
	-	1085	1227	142*	-3.408
Stenen 1	1515	-	1852	337**	-5.481
Semans	957	-	1220	263**	-7.31
Medstead	410	542	-	132*	-3.32
	410	-	892	482**	-7.29
	-	542	892	350**	-5.51
Stenen 2	1580	-	1837	257*	-3.36

[a] Oven dry basis
*, ** Significant at $P \leq 0.05$ and 0.01, respectively as determined by *t* test.

Spore populations were also determined at harvest in 1989 on fields which had been cropped to wheat and barley (Table 13). It was observed that VAM spore numbers had increased significantly from the values obtained at seeding, indicating that the host crops grown in 1989 had a favorable effect on the spore numbers. Despite the increase in spore numbers during the 1989 growing season, the reduction in VAM spores associated with canola and summerfallow from the previous year, remained evident at harvest.

At the various sites in the different soil zones, it was observed infection of wheat was reduced when preceded by a year of either summerfallow or wheat (Fig. 7). Similarly, percent VAM infection of in barley grown in adjacent fields was reduced if canola had been grown in the previous year or if the field had been fallowed in the previous year (Fig. 8). This reduction was more conspicuous at the second and third sampling time at most of the sites. Canola caused a greater reduction in the percent VAM infection than did summerfallow in both wheat and barley.

The occurrence of eight VAM species identified from the soil collected in 1989 is illustrated in Table 14. *Glomus* species were the most abundant in the different Saskatchewan soils. *Acaulospora* and *Gigaspora* spores were also frequently observed in the different soils, but these were apparently from only one species. *G. mosseae* and *G. geosporum* occurred in all of the soil.

Table 13. Increase in spore number due to wheat and barley grown in fields previously cropped to canola and wheat or kept summerfallow

Site	1988 crop	1989 crop	Spore No. at seeding	Spore No. at harvest	Increase in Spore No.	*t* value
			per 100 g soil[a]			
Lucky lake	Wheat	Wheat	1788	2105	317**	-5.62
	Summerfallow	Wheat	1535	1805	270**	-5.18
Watrous	Wheat	Wheat	1228	1468	240**	-5.46
	Summerfallow	Wheat	1058	1193	108*	-2.61
	Canola	Wheat	950	1108	158*	-3.51
Stenen	Wheat	Wheat	1852	2113	261*	-2.93
	Canola	Wheat	1515	1853	338*	-3.37
Semans	Wheat	Barley	1220	1638	418**	-9.21
	Canola	Barley	958	1213	255*	-3.95
Medstead	Wheat	Barley	893	1090	197[ns]	-1.92
	Summerfallow	Barley	543	800	257**	-5.72
	Canola	Barley	410	623	213[ns]	-2.35
Stenen	Wheat	Barley	1839	2145	306*	-2.85
	Canola	Barley	1580	1873	293*	-3.04

[a] Oven dry basis
*, ** Significant at $P \leq 0.05$ and 0.01, respectively; ns = not significant as determined by *t* test.

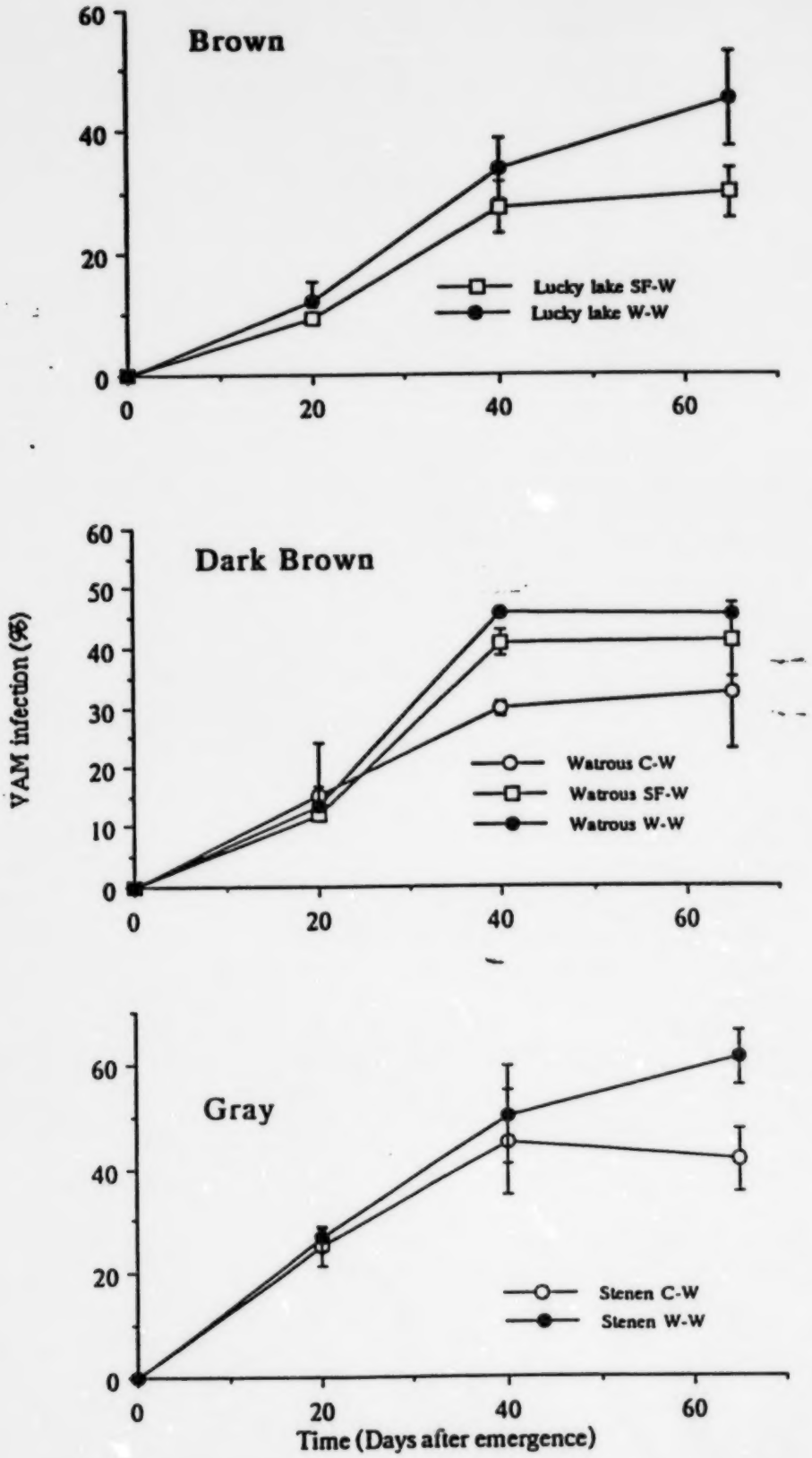

Figure 7. Effect of crop rotations including wheat (W), summerfallow (SF), and canola (C) on percent VAM infection of wheat.

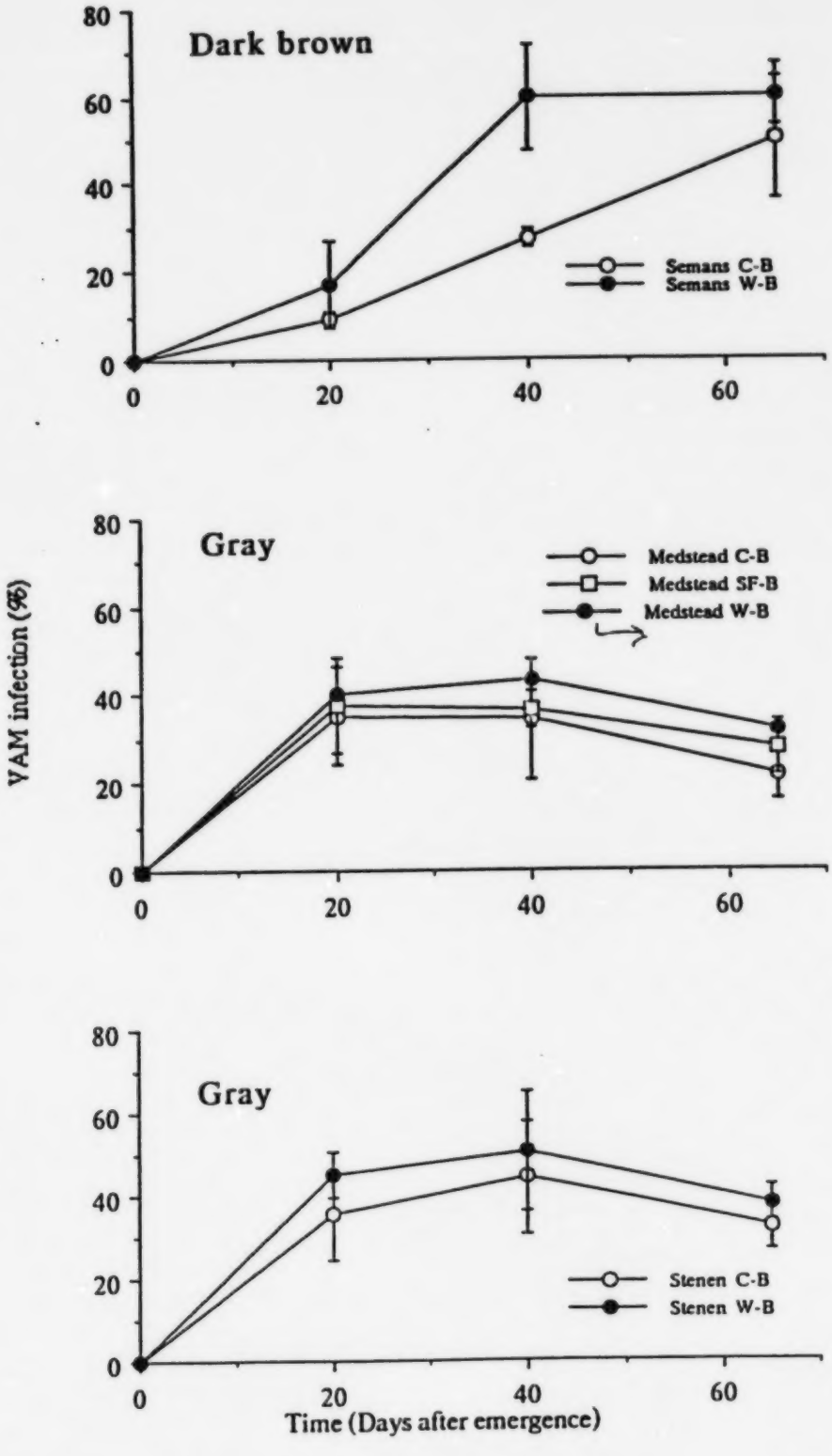

Figure 8. Effect of crop rotations including summerfallow (SF) and canola (C) on percent VAM infection of barley

Table 14. Presence of VAM spores.

VAM spores	Soil					
	Outlook	Semans	Englefeld	Glenavon	Stenen	Medstead
Acaulospora sp.	•	•	•	•	o	•
Gigaspora margarita	•	•	•	o	•	•
Glomus caledonium	•	•	o	o	o	o
G. fasciculatum	•	o	o	o	o	•
G. geosporum	•	•	•	•	•	•
G. mosseae	•	•	•	•	•	•
G. pallidum	•	•	o	o	•	o
G. versiforme	o	•	•	o	o	o

Note: • = Present; o = Absent

3.1.3 1989 Field Study - The Effect of Long-term Crop Rotations and Fertilizer Application on VAM Infection of Spring Wheat.

The effects of long-term crop rotations and fertilizer application on the percent VAM infection of spring wheat roots at Swift Current are reported in Table 15.

Table 15. Effects of long-term crop rotations on percent VAM infection of spring wheat roots at Swift Current, 1989.

Treatment (rotation)	Days after Planting (DAP)			
	25	45	65	85
continuous wheat (fert.)	14.3	13.0	16.3	18.0
SF - ***WH***(fert.)	6.0	14.7	21.3	25.7
SF - ***WH*** - WH (fert.)	10.0	11.0	32.3	32.3
SF - WH - ***WH*** (fert.)	7.7	13.3	22.3	31.7
SF - ***WH*** - WH (unfert.)	11.0	28.0	48.3	58.3
SF - WH - ***WH*** (unfert.)	24.0	45.0	55.7	68.0
LSD (p = 0.05)	9.3	13.6	17.8	16.5

Regardless of treatment, percent VAM infection was observed to increase throughout the growing season, reaching a maximum of 68 % VAM infection at 85 days after planting in wheat grown in the third year of a three year unfertilized summerfallow - wheat - wheat rotation. At each sampling date, maximum levels of VAM infection were achieved in wheat grown in the unfertilized three year summerfallow - wheat - wheat rotation. Moreover, the highest levels of VAM infection were achieved in the third year of this unfertilized rotation although VAM infection was not significantly different from the levels achieved in the second year of the rotation at the third and fourth sampling period. Thus, inclusion of a summerfallow year in this rotation appears to have reduced VAM infection in the year immediately following the summerfallow year; however, any adverse effects of a summerfallow year on VAM infection were apparently overcome, as evidenced by the nonsignificant differences in VAM infection at the third and fourth sampling period. It is likely that reduced VAM infection resulted as a consequence of a reduction in the number of infective propagules present in the soil during the summerfallow year. VA mycorrhizae are dependent on the presence of a host plant for growth and spore production. Spores germinating in the absence of an appropriate host will eventually exhaust reserves and die out. Because host plants are not present during a summerfallow year, spore numbers and infective propagules can only decline. Thus levels of VAM infection were reduced during the summerfallow year and the infection in the subsequent crop was reduced; however, as the growing season progressed, data suggest that the number of infective propagules were restored. Thus, high levels of infection were achieved in the third year of the rotation.

In light of the fact that VA mycorrhizae require a suitable host plant to produce infective propagules, one would expect that the maximum levels of infection would be

observed in the continuous wheat. Contrary to expectations, it was observed that VAM infection in the continuous wheat rotation were generally the lowest, although these levels were not statistically lower than either the fertilized two year summerfallow wheat rotation or the fertilized three year summerfallow - wheat - wheat rotation. Two factors must be considered: 1) the continuous wheat rotation received 17.1 kg.ha-1 of seed placed P_2O_5 on an annual basis and, according to several researchers, the application of fertilizer P can lead to a reduction in the level of VAM infection; and 2) droughty conditions persisted through much of the 1989 growing season and thus crops grown on summerfallow may have been healthier and more vigorous due to improved moisture status of the soil as compared to crops grown on stubble. VAM are dependant on the host plant for their carbohydrate supply and thus a healthier plant may encourage healthier symbiotic association with the VAM. Thus the generally low levels of VAM infection observed in the continuous wheat rotation may reflect both a high nutrient status and a relatively low moisture status in the soil.

A comparison of the levels of VAM infection in the fertilized and the unfertilized three year summerfallow - wheat - wheat rotation clearly demonstrates the negative effect that fertilizer application had on VAM infection. In both the second and third year of this rotation, levels of VAM infection were generally highest on the unfertilized rotation. Significant reductions in VAM infection were particularly evident in the third year of the rotation. In the second year of the rotation, although VAM infection levels appeared to be reduced in the fertilized rotation, reductions were statistically significant only at the second and fourth sampling time.

The numbers of spores present in the soil in the spring of 1989 at Swift Current reflects many of the same trends observed for percent VAM infection (Table 16). It should be noted that the number of spores present in the soil in the spring of 1989, reflects the effect that the previous crop grown in the previous year had on the production of spores.

At Swift Current, spore numbers tended to be greatest in the unfertilized three year summerfallow - wheat - wheat rotation. Moreover, the number of spores in this rotation was less in the year following the summerfallow year than in the third year of the rotation (i.e., wheat grown on stubble).. Thus it appears that the number of spores in this rotation was reduced during the summerfallow year. A similar trend exists in the fertilized three year summerfallow - wheat - wheat rotation although the reduction associated with the summerfallow year was not statistically significant. No statistically significant differences in the number of spores existed between those treatments which received fertilizer application. Thus, it was concluded that at the Swift Current location, the number of spores present in the soil was strongly influenced by the level of fertilizer application.

Table 16. The effect of long term fertility treatments and rotations on spore numbers in the spring of 1989 at Swift Current, Indian Head, Scott and Melfort.

Treatment[a] (rotation)	Swift Current	Indian Head	Scott	Melfort
	Number of spores / 50 grams soil			
cont. ***WH*** (fert.)	136	442	327	330
cont. ***WH*** (unfert.)	NA[b]	605	NA	225
SF - ***WH*** (fert.)	116	243	120	146
SF - ***WH*** (unfert.)	NA	337	NA	NA
SF - ***WH*** - WH (fert.)	129	298	102	100
SF - WH -***WH*** (fert.)	148	442	76	125
SF - ***WH*** - WH(unfert.)	186	375	NA	190
SF - WH - ***WH*** (unfert.)	264	511	NA	171
SF - CN - WH (fert.)	NA	NA	195	62
SF - CN - ***WH*** -WH (fert.)	NA	NA	NA	65
SF - CN - WH - ***WH*** (fert.)	NA	NA	NA	96

a - SF denotes summer fallow, WH denotes spring wheat, CN denotes canola. The bolded and italicized letters denote the treatment and year of the rotation.
b - denotes "Not Available".

At the Indian Head location, it was also observed that levels of VAM infection were relatively high and that infection generally increased over the growing season (Table 17). The effect of fertilizer application on levels of VAM infection was clearly evident when fertilized and unfertilized continuous wheat rotations and the two year summerfallow - wheat rotation were compared. In both of these rotations, application of fertilizer (83 $kg.ha^{-1}$ N and 25 kg ha^{-1} P_2O_5) lead to reductions in the levels of VAM infection, although these reductions were not significant in the two year rotation at the first two sampling dates. In contrast, essentially no difference in the level of VAM infection existed between the fertilized and unfertilized second year of the three year summerfallow - wheat - wheat rotation. In the third year of this rotation, data suggest that fertilizer application may have reduced levels of VAM infection although these reductions were not statistically significant. It is not known why this discrepancy existed.

The negative effect of fertilizer application on spores numbers was particularly apparent at Indian Head (Table 16). It was observed that for each rotation, application of fertilizer in 1988 led to a reduction in the number of spores in the spring of 1989. Thus reduced levels of VAM infection observed at Indian Head may reflect both a reduced number of spores present in the fertilized treatments and the inhibitory effect of fertilizer application on the processes of infection and subsequent hyphal development.

Table 17. Effects of long-term crop rotations on percent VAM infection of spring wheat roots at Indian Head, 1989.

Treatment (rotation)	Days after Planting (DAP)			
	18	40	60	80
continuous wheat (fert.)	34.3	35.8	37.5	59.5
continuous wheat (unfert.)	35.5	57.5	56.0	71.0
SF - ***WH*** (fert.)	12.5	32.5	25.3	38.3
SF - ***WH*** (unfert.)	14.0	42.5	51.5	49.3
SF - ***WH*** - WH (fert.)	24.4	28.0	29.3	51.8
SF - WH - ***WH*** (fert.)	33.5	32.5	31.5	52.0
SF - ***WH*** - WH (unfert.)	23.8	32.0	31.8	46.5
SF - WH - ***WH*** (unfert.)	29.8	39.5	48.5	61.0
LSD (p = 0.05)	12.2	15.7	13.3	13.5

In contrast to results observed at Swift Current, levels of VAM infection at Indian Head were generally reduced in wheat grown on summerfallow as compared to wheat grown in a continuous wheat rotation, particularly when comparisons are made between treatments receiving similar fertilizer regimes (Table 17). It is possible that a more favorable moisture regime at the Indian Head location allowed for adequate wheat growth in the continuous wheat plots, thus encouraging the development and production of infective VAM propagules. The number of spores present in the soil at Indian Head in the spring of 1989 suggest that continuous wheat encouraged the development of spores (Table 16). When comparisons were made between rotations receiving similar fertilizer regimes, the continuous wheat rotation consistently had the highest number of VAM spores in the soil, although the apparent differences were not always statistically significant. Thus, decreases in VAM infection associated with the inclusion of summerfallow in the rotations at Indian Head likely reflect the influence that the summerfallow year had on the numbers of spores, and thus infective propagules, present in the soil.

Levels of VAM infection at Scott were relatively low, perhaps reflecting the relatively high rates of seed placed P_2O_5 applied to all rotations at this location (i.e., 25 - 31.6 kg ha^{-1} P_2O_5) (Table 18). In comparing the levels of VAM infection in the continuous wheat rotation to that in the two year summerfallow - wheat rotation, data indicate that the inclusion of the summerfallow year reduced levels of VAM infection. These reduction were statistically significant, however, only at the first sampling date. This suggests that the numbers of infective propagules were able to recover to some degree during the first portion of the growing season. Although not statistically significant, a similar reduction is apparent when comparing the first two sampling dates of the

continuous wheat rotation to the second year of the three year summerfallow - wheat - wheat rotation. Again, levels of VAM infection appear to have recovered by day 67. It is particularly interesting to note that levels of VAM infection were generally higher in the third year of the summerfallow - wheat - wheat rotation than in the continuous wheat rotation, although this was statistically significant only at the fourth sampling period. It is not known why this rotation apparently favored the levels of VAM infection.

Table 18. Effects of long-term crop rotations on percent VAM infection of spring wheat roots at Scott, 1989.

Treatment (rotation)	Days after Planting (DAP)			
	27	47	67	89
continuous wheat (fert.)	21.0	11.5	21.8	19.8
SF - ***WH*** (fert.)	11.8	5.3	18.0	14.8
SF - ***WH*** - WH (fert.)	13.5	5.8	23.5	21.0
SF - WH - ***WH*** (fert.)	18.8	16.8	37.8	33.8
SF - CN - ***WH*** (fert.)	19.8	9.0	23.5	23.0
LSD (p = 0.05)	7.9	8.4	16.4	13.6

The numbers of spores present in the soil in the spring of 1989 at Scott indicate that inclusion of a summerfallow year reduced the number of spores present in the soil in all the rotations (Table 16). It is particularly interesting to note that the number of VAM spores present in the three year rotation was considerable lower than the number observed in the continuous wheat rotation. One might expect higher levels of infection in the continuous wheat; however, it was observed that the levels of infection were generally higher in the third year of the summerfallow - wheat - wheat rotation than in the continuous wheat rotation, although this was statistically significant only at the fourth sampling period. This result suggests that some factor, other than the presence of infective propagules was responsible for this unexpected result.

Because canola is a non-host crop, it was expected that the three year summerfallow - canola - wheat rotation would be associated with particularly low levels of VAM infection. In contrast to this expectation, however, the levels of VAM infection in this rotation were very similar to those observed in the continuous wheat rotation. Furthermore, the number of spores present in this rotation was significantly higher than in any other rotation which included a single summerfallow year. The number of spores present following the canola year was not, however, as high as the number present in the continuous wheat rotation. It is not known why the inclusion of canola, a non-host crop, did not result in a further reduction in the number of spores present in the soil.

Lack of statistically significant reductions in VAM infection associated with the inclusion of summerfallow and canola in crop rotations at Scott may be a consequence of the relatively high levels of seed-placed fertilized P applied to all rotations. This seed-placed fertilizer may have interfered with the initial infection and subsequent hyphal

development of VAM in the wheat roots. Thus levels of VAM infection were reduced to such an extent that the the majority of effects associated with crop rotation were essentially obliterated, regardless of the effects of rotation on the presence of VAM spores.

The effect of long - term crop rotations on percent VAM infection of spring wheat roots at Melfort is reported in Table 19. A great deal of variability was associated with the results obtained at the Melfort site and, as a consequence, LSD values tended to be quite high. Despite relatively high variability, it was observed that application of fertilizer generally reduced the levels of VAM infection in similar rotations. Thus, when comparing the fertilized and unfertilized continuous wheat rotation, the highest levels of VAM infection were achieved on the unfertilized rotation, although this was statistically significant only at the third and fourth sampling period. Similarly, when comparing the fertilized versus the unfertilized three year summerfallow - wheat - wheat rotation , it was observed that in the second year of this rotation levels of VAM infection were consistently higher in the unfertilized treatments, although not significantly so during the third and fourth sampling period. In the third year of this rotation, however, levels of VAM infection appeared relatively unaffected by the application of fertilizer. It is not known why the application of fertilizer did not reduce VAM infection in the third year of this rotation although perhaps lack of significant differences merely reflects the large degree of variability encountered at this location.

Table 19. Effects of long-term crop rotations on percent VAM infection of spring wheat roots at Melfort, 1989.

Treatment (rotation)	Days after Planting (DAP)			
	27	47	67	88
continuous wheat (fert.)	34.3	17.0	24.5	29.0
continuous wheat (unfert.)	35.8	38.3	57.0	63.8
SF - ***WH*** (fert.)	17.3	15.0	35.5	46.3
SF - ***WH*** - WH (fert.)	11.3	28.3	35.5	38.3
SF - WH - ***WH*** (fert.)	33.5	30.8	26.0	39.8
SF - ***WH*** - WH (unfert.)	31.0	52.5	45.5	52.3
SF - WH - ***WH*** (unfert.)	22.8	33.5	41.0	37.3
SF - CN - ***WH*** (fert.)	14.5	26.5	24.3	41.0
SF - CN - ***WH*** - WH (fert.)	9.5	28.5	20.0	28.0
SF - CN - WH - ***WH*** (fert.)	21.0	20.5	23.8	23.5
LSD (p = 0.05)	15.9	21.4	17.6	17.6

Similar trends were observed for the number of spores present in the soil at Melfort during the spring of 1989 (Table 16). Despite a great deal of variability and a subsequently high LSD value, it was observed that application of fertilizer generally reduced the numbers of spores present in similar rotations. Thus, reductions in VAM infection may result from the effect of fertilizer application on the production of infective propagules, and (or) the effect of fertilizer application on the infection process itself.

A comparison of the levels of VAM infection in the fertilized continuous wheat versus the fertilized two year summerfallow - wheat rotation indicates that VAM infection was reduced by the inclusion of the summerfallow year only at the first sampling date (Table 19). At the remaining sampling dates, the inclusion of the summerfallow year did not significantly influence the level of VAM infection. In fact, data suggest that the summerfallow year may have enhanced VAM infection at the third and fourth sampling dates although this enhancement was not statistically significant. Similarly, comparison of the fertilized continuous wheat rotation with the second year of the fertilized three year summerfallow - wheat - wheat rotation indicated that the levels of VAM infection were significantly reduced in the wheat crop grown on summerfallow at the first sampling date whereas at later sampling dates the levels of infection had recovered in the wheat grown on summerfallow and may even have been greater than those achieved in the continuous wheat rotation, although not significantly so. Although not statistically significant, it is interesting to note that in both the fertilized and unfertilized three year summerfallow - wheat - wheat rotations, the highest levels of VAM infection were often achieved in the wheat grown on summerfallow, rather than in wheat grown on stubble. As was observed at Swift Current, droughty conditions persisted at the Melfort site and it is possible that wheat grown on summerfallow benefitted from the increased moisture status of the soil and thus plants tended to be healthier, encouraging VAM development.

Similar trends were observed for the number of spores present in the soil during the spring of 1989 at Melfort (Table 16). It was observed that the numbers of spores present in the two year summerfallow wheat rotation were reduced as compared to the continuous wheat rotation, although this reduction was not statistically significant. Similarly, inclusion of a summerfallow year in a three year summerfallow - wheat - wheat rotation reduced the numbers of spores present in the soil although these reductions were not statistically significant.

It was anticipated that inclusion of canola in the rotations would lead to a reduction in the levels of VAM infection. With the exception , however, of the first sampling date of the three year summerfallow - canola - wheat rotation and the four year summerfallow - canola - wheat - wheat rotation, the levels of VAM in these rotations did not differ significantly from levels achieved in the continuous wheat rotations. The numbers of spores present in the rotation including canola were extremely low as compared to other rotations at this site and one might have expected relatively low levels of infection as a consequence of the low numbers of infective propagules (Table 16). These reductions in VAM infection, however, were not observed. As was suggested for the canola rotations at Scott, lack of significant reductions associated with the inclusion of the canola in the rotation may reflect the relatively high rates of phosphorus applied to these rotations (31 kg ha^{-1} P_2O_5) as well as the unfavorable soil moisture conditions.

Results indicate that fertilizer application had a pronounced effect on the levels of VAM infection in spring wheat roots at all experimental locations. These results are in keeping with a number of studies which have indicated that both phosphorus (Hayman, 1970; Daft and Nicolson, 1969; Ross 1971) and nitrogen (Porter and Beute, 1972; Hayman, 1975) may significantly reduce root colonization. For example, at both Indian

Head and Melfort, fertilization of continuous wheat generally reduced infection (Fig.9). It is interesting to note that levels of VAM infection at both sites were not affected by fertilizer application at the earliest sampling period and that statistically significant reductions in VAM infection were observed at later sampling periods. This suggests that although early infective stages were relatively unaffected by fertilizer application, subsequent colonization of roots was impeded in the presence of high levels of fertilizer. Moreover, fertilizer application likely increased total root length, and it is possible that root length increased more rapidly than VAM infection and colonization, resulting in an apparent decline in the levels of VAM infection.

Similar results were observed at Swift Current in both the second and third year of a three year summerfallow - wheat - wheat rotation (Fig. 10). In the second year of the rotation, VAM infection was unaffected by fertilizer application at the first sampling period (Fig. 10a). Although statistically significant only at the final sampling period, data suggest that VAM infection was reduced at the subsequent sampling periods by fertilizer application. In the third year of the rotation, however, VAM infection was significantly reduced by the application of fertilizer at all sampling dates (Fig. 10b). It is of interest to note that very little fertilizer N was applied at Swift Current to the fertilized treatments (i.e. 3.7 kg N ha^{-1} was applied to all but one treatment) which suggests that the reductions associated with fertilizer application were primarily associated with application of phosphate fertilizer.

Crop rotation was also observed to significantly affect the level of VAM infection in spring wheat. For example, at Indian Head, levels of VAM infection in the unfertilized continuous wheat rotation were generally higher that the levels of VAM infection in wheat grown on summerfallow (Fig. 11). Thus inclusion of summerfallow in the rotation led to reduced levels of VAM infection in the subsequent crop. It is likely that reduced VAM infection resulted as a consequence of a reduction in the number of infective propagules present in the soil during the summerfallow year. VA mycorrhizae are dependent on the presence of a host crop for growth and spore production. Spores germinating in the absence of a suitable host will eventually exhaust reserves and die out.

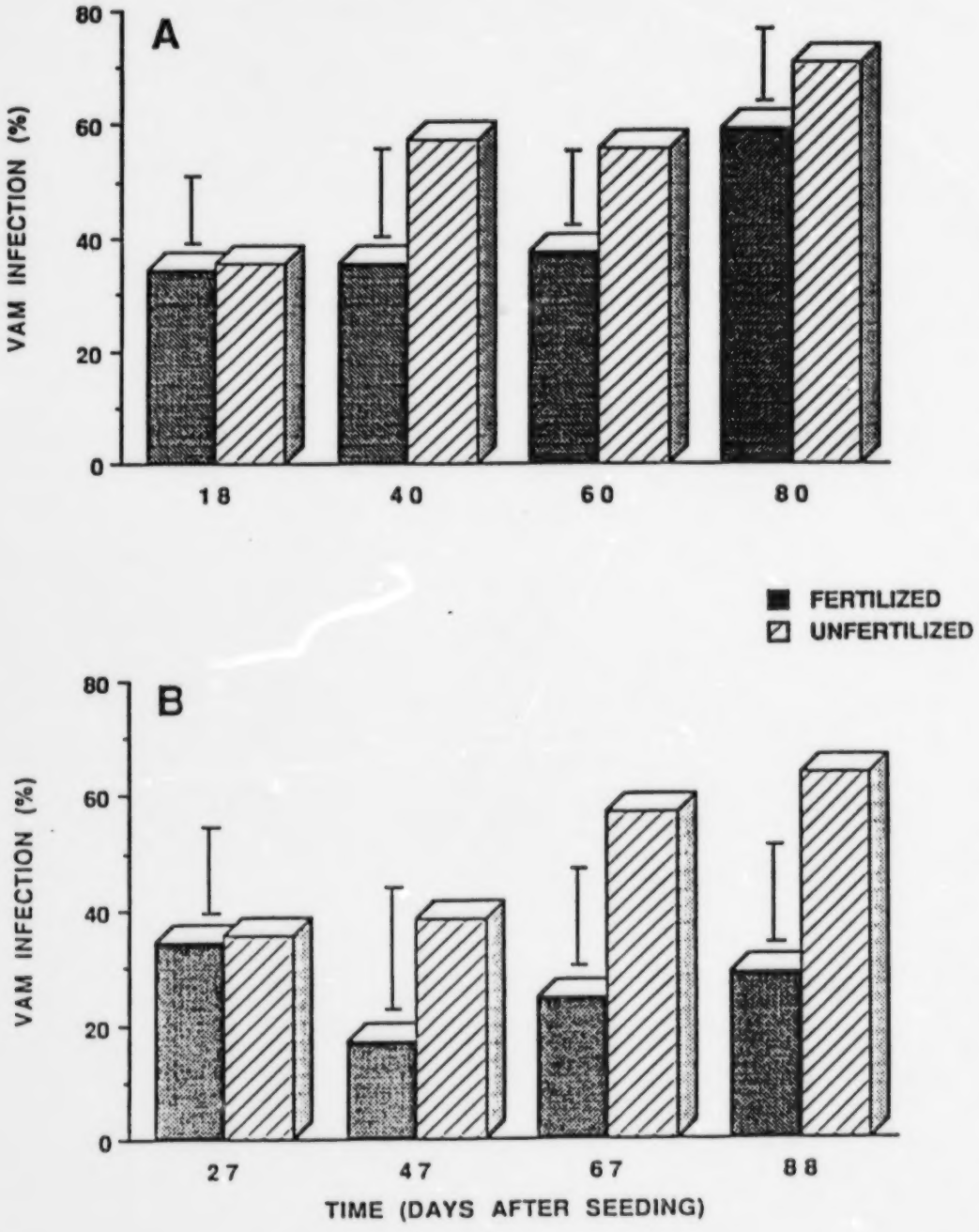

Figure 9. The effect of fertilizer application on percent VAM infection of spring wheat grown in a continuous rotation at Indian Head (A) and Melfort (B).

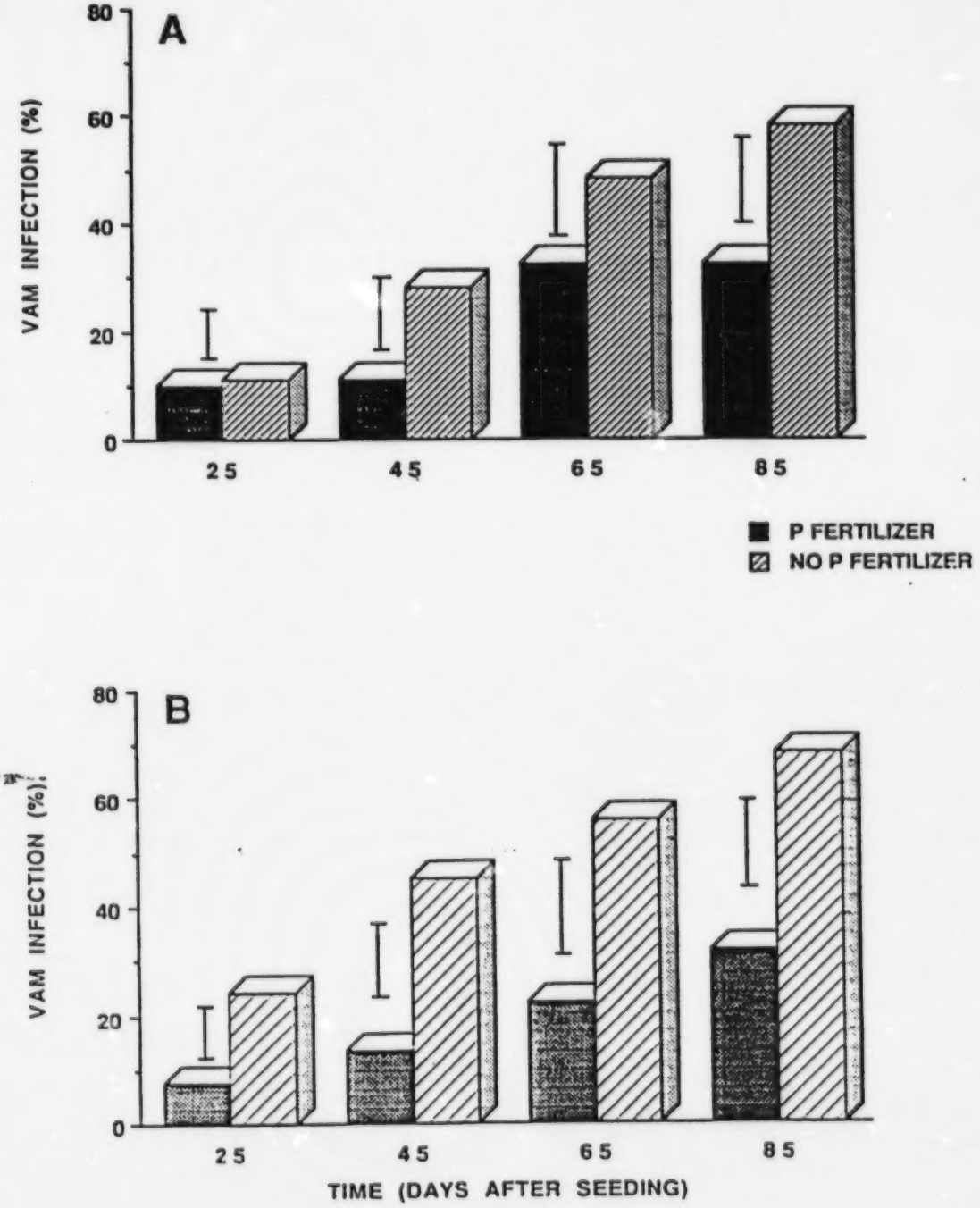

Figure 10. The effect of fertilizer application on percent VAM infection of spring wheat in the second (A) and third (B) year of a three year summerfallow - wheat - wheat rotation at Swift Current.

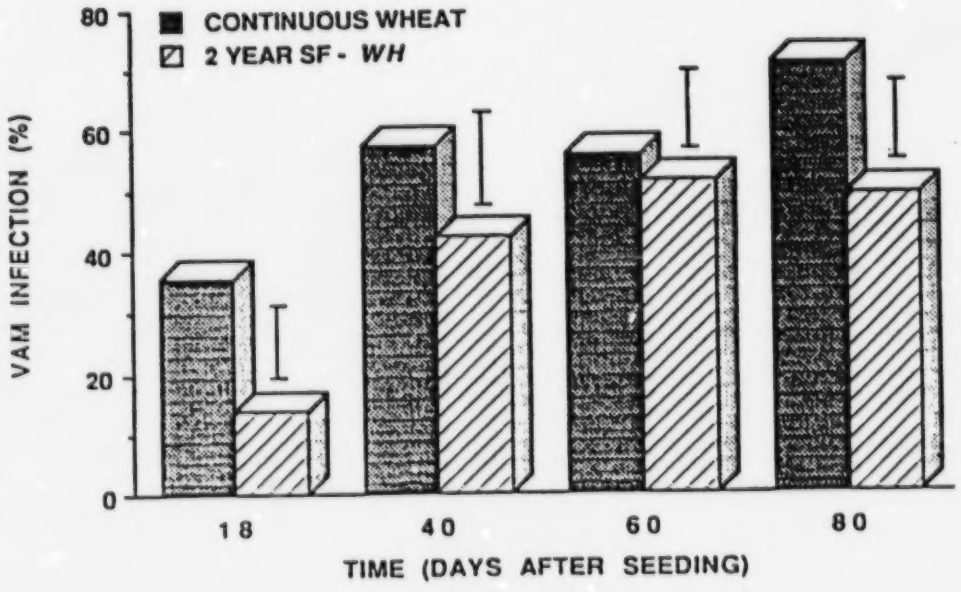

Figure 11. The effect of summerfallow on percent VAM infection of unfertilized spring wheat grown in a two year summerfallow - wheat rotation at Indian Head.

Comparison of the levels of infection in the continuous wheat rotation versus levels of VAM infection in the second and third year of the summerfallow - wheat - wheat rotation at Indian Head indicate a similar trend (Fig. 12a). VAM infection was reduced in the second year of the rotation (i.e., wheat grown on summerfallow) as compared to the continuous wheat rotation. Levels of VAM infection did, however, recover somewhat in the third year of the rotation although data suggest that even in the third year of the rotation, levels of VAM infection continued to be reduced by the preceding summerfallow year. These trends were not observed, however, where fertilizer had been applied (Fig. 12b), suggesting that fertilizer application suppressed VAM infection in all treatment to such a degree that differences in VAM infection associated with crop rotation were essentially obliterated.

Inclusion of canola in a rotation would be expected to similarly reduce levels of VAM infection in subsequent crops because canola is a non-host crop. Results from Melfort (Fig. 13) and Scott (Fig. 14) , however, indicate that inclusion of canola in the rotation had little or no effect on the level of VAM infection in subsequent crops. It is important to note that at both Scott and Melfort, these rotations received relatively high levels of both nitrogen and phosphorus and thus, it is probable that the effect of the high fertilizer rates obliterated any rotation effects. Indeed, VAM infection on these fertilized rotations tended to be very low, regardless of the rotation treatment (i.e., 10 - 35 % VAM infection).

Results from this study indicate that VAM infection is influenced both by fertilizer application and crop rotation. It is not known, however, if the effects of fertilizer application and crop rotation are the result of a direct effect of these factors on VA mycorrhizae, or as a consequence of an indirect effect . For example, fertilizer application and crop rotation may influence crop development and (or) the population of soil organisms in general, which may in turn have a subsequent indirect effect on the infectivity of VA mycorrhizae.

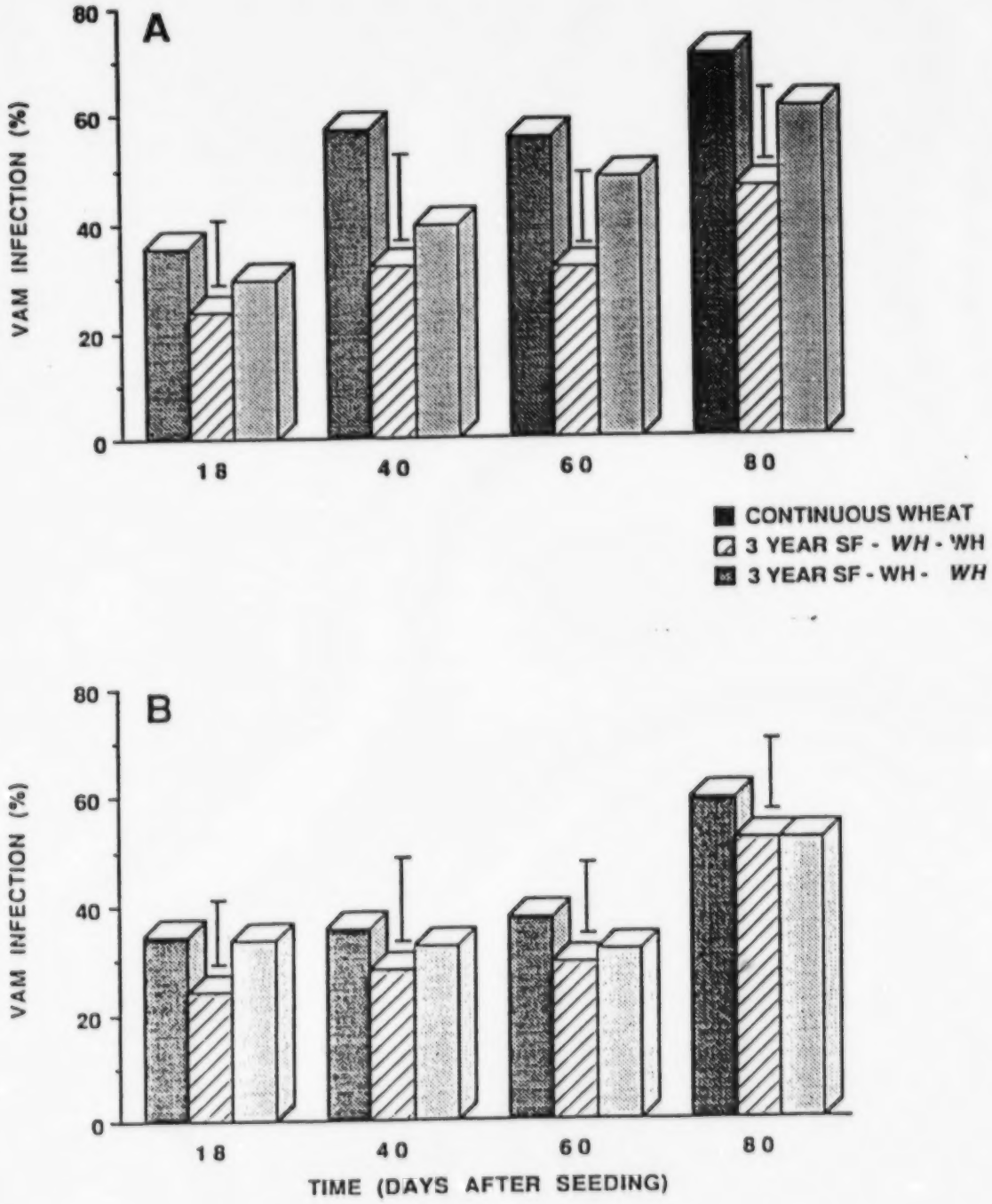

Figure 12. Effect of summerfallow on infection of spring wheat grown in an unfertilized (A) and fertilized (B) three year summerfallow - wheat - wheat rotation at Indian Head.

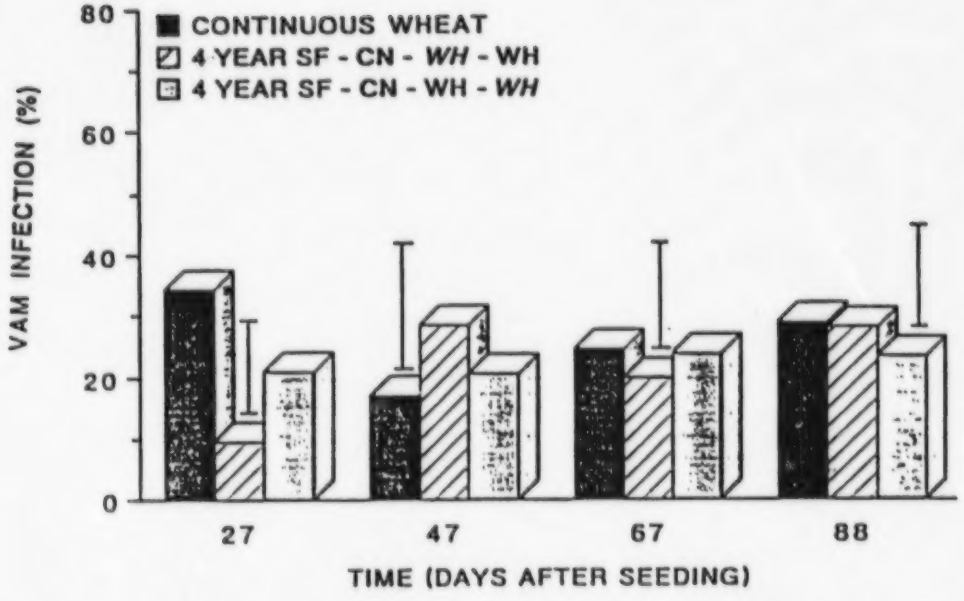

Figure 13. Effect of canola on VAM infection of spring wheat grown in a four year summerfallow - canola - wheat - wheat rotation at Melfort.

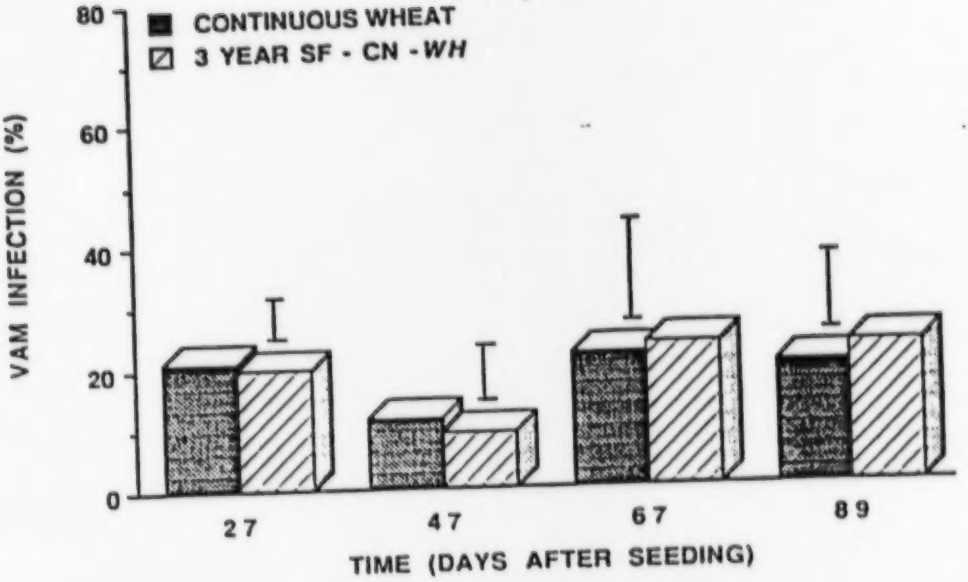

Figure 14. Effect of canola on VAM infection of spring wheat grown in a three year summerfallow - canola - wheat rotation at Scott.

3.1.4 1989 Field Study - The Effect of Herbicide Application on VAM Infection of Spring Wheat.

A field study was conducted at Loon Lake to determine the effect of long-term herbicide application on VAM infection of spring wheat. At the first sampling date, it was observed that application of Hoegrass and Glean favoured the development of VAM infection wheat compared to the remaining herbicide treatments (Table 20). The lowest levels of VAM infection were associated with the application of Hoegrass/Torch tank mix.

Table 20. Percent VAM infection of spring wheat roots at Loon Lake, 20 days after planting.

Herbicide	Fertilizer Treatment (lb acre^{-1})		MEAN
	Unfertilized	80 N, 40 P_2O_5	
untreated	45.0	21.2	33.1b*
Hoegrass and Glean	35.5	45.2	40.4a
Hoegrass / Torch	20.5	21.0	20.8c
Hoegrass and Buctril M	26.8	30.0	28.4b
MEAN	32.0a	29.4a	

* - means followed by the same letter in a column or row are not significantly different at the 5% level of probability (Duncan's Multiple Range Test)

Similarly, at the second sampling date, it was observed that the treatment receiving Hoegrass and Glean had the highest levels of infection although not significantly higher than Hoegrass and Bucril M (Table 21). It is particularly interesting that Hoegrass and Glean (unfertilized) had relatively high levels of VAM infection at this sampling date. This result suggests that this herbicide combination may have actually stimulated VAM infection.

Table 21 . Percent VAM infection of spring wheat roots at Loon Lake, 40 days after planting.

Herbicide	Fertilizer Treatment (lb acre^{-1})		MEAN
	Unfertilized	80 N, 40 P_2O_5	
untreated	31.8	22.8	27.3c
Hoegrass and Glean	56.2	30.8	43.5a
Hoegrass / Torch	33.2	31.0	32.1bc
Hoegrass and Buctril M	31.8	44.5	38.1ab
MEAN	38.2a	32.3a	

* - means followed by the same letter in a column or row are not significantly different at the 5% level of probability (Duncan's Multiple Range Test)

At the third sampling date, the Hoegrass and Glean treatment again had the highest level of VAM infection although this level of VAM infection was not significantly different than the remaining herbicide treatments (Table 22). Furthermore, it was observed that there were significant differences between the fertilized and the unfertilized treatments.

Table 22. Percent VAM infection of spring wheat roots at Loon Lake, 60 days after planting.

Herbicide	Fertilizer Treatment (lb acre^{-1})		MEAN
	Unfertilized	80 N, 40 P_2O_5	
untreated	39.2	26.8	33.0a
Hoegrass and Glean	58.0	31.0	44.5a
Hoegrass / Torch	44.0	32.5	38.2a
Hoegrass and Buctril M	37.5	34.0	35.8a
MEAN	44.7a	31.1b	

* - means followed by the same letter in a column or row are not significantly different at the 5% level of probability (Duncan's Multiple Range Test)

Finally, at the fourth sampling date, the Hoegrass and Glean treatment again had the highest level of VAM infection although this level of VAM infection was not significantly different than the remaining unfertilized herbicide treatments (Table 23). The lowest levels of VAM infection were associated with the fertilized treatments.

Table 23. Percent VAM infection of spring wheat roots at Loon Lake, 81 days after planting.

Herbicide	Fertilizer Treatment (lb acre^{-1})		MEAN
	Unfertilized	80 N, 40 P_2O_5	
untreated	56.5	21.2	38.8a
Hoegrass and Glean	58.5	43.0	50.8a
Hoegrass / Torch	47.5	34.5	41.0a
Hoegrass and Buctril M	56.8	38.8	47.8a
MEAN	54.8a	34.4b	

* - means followed by the same letter in a column or row are not significantly different at the 5% level of probability (Duncan's Multiple Range Test)

The effect of herbicide application on the number of spores in the spring of 1989 is reported in Table 24. Data suggest that long-term herbicide application had little effect on spore numbers. In contrast, long-term application of fertilizer at relatively high rates led to a reduction in the number of spores present in the soil. Thus reduced levels of VAM infection in fertilized treatments may reflect the reduction in infective spores present in the soil during the early growing season and (or) a reduction in the level of infectivity of these spores associated with a direct effect of the applied fertilizer.

The results from this study data suggest that both fertilizer and herbicide application may be important factors influencing the levels of VAM infection in spring wheat. The effect of herbicide application were generally more prevalent during the early portion of the growing season whereas fertilizer application appeared to influence VAM infection during the latter portion of the growing season. Although results suggest that herbicide application may influence VAM infection, it is not known whether the herbicides directly affected the VAM, or if herbicide application altered crop growth, indirectly affecting the VAM. Alternatively, herbicide application may have influenced VAM infection through alteration of the weed spectrum associated with the different herbicides. Although many weeds are readily infected by VAM, some are resistant; thus, the presence of non-host weeds may have altered the subsequent production of VAM spores.

Table 24. Effect of herbicide and fertilizer application on the number of spores at Loon Lake, 1989.

Herbicide	Fertilizer Treatment (lb acre-1)		MEAN
	Unfertilized	80 N, 40 P2O5	
untreated	330	176	253
Hoegrass and Glean	348	199	274
Hoegrass / Torch	289	198	243
Hoegrass and Buctril M	306	148	227
MEAN	318	180	

3.2 Growth Chamber Studies

3.2.1 Growth Chamber Experiment #1 - The Development of VAM Infection in Wheat, Lentil and Field Pea.

VAM infection of wheat, lentil and field pea roots was observed at early plant development stages. As early as 20 days after seeding, percent infection of wheat, lentil and field pea had reached levels of 25, 23 and 15 percent, respectively (Fig. 15a). Infection of lentil and field pea roots was similar both in terms of degree of infection and pattern of infection. In both crops, a gradual increase in the degree of infection between days 20 and 30 was followed by a rapid increase in the degree of infection between days 30 and 40. Following day 40, the percent infection of lentil and field pea roots remained relatively unchanged.

Infection of wheat roots did not follow the same pattern as that observed for lentil and field pea. Increases in the percent infection of wheat roots occurred at a relatively constant rate over the growing period and progressed at a slower rate than that observed in both lentil and field pea. Furthermore, at all but the first harvest, the level of infection of wheat was less than that observed in either lentil or field pea. Although percent infection was generally less in wheat than in either of the legume crops, wheat root length exceeded the root length of both lentil and field pea at all but the first sampling period (Fig. 16a). As a consequence, although percent infection of wheat roots was less than either lentil or field pea, the actual length of root infected by VAM was greatest for wheat (Fig. 16b). This is an important finding as it demonstrates that the relative importance of VAM infection in crop production and nutrition may not be related simply to degree of infection; rather, the actual length of infected root may be a more representative indicator of the importance of VAM infection in any given crop.

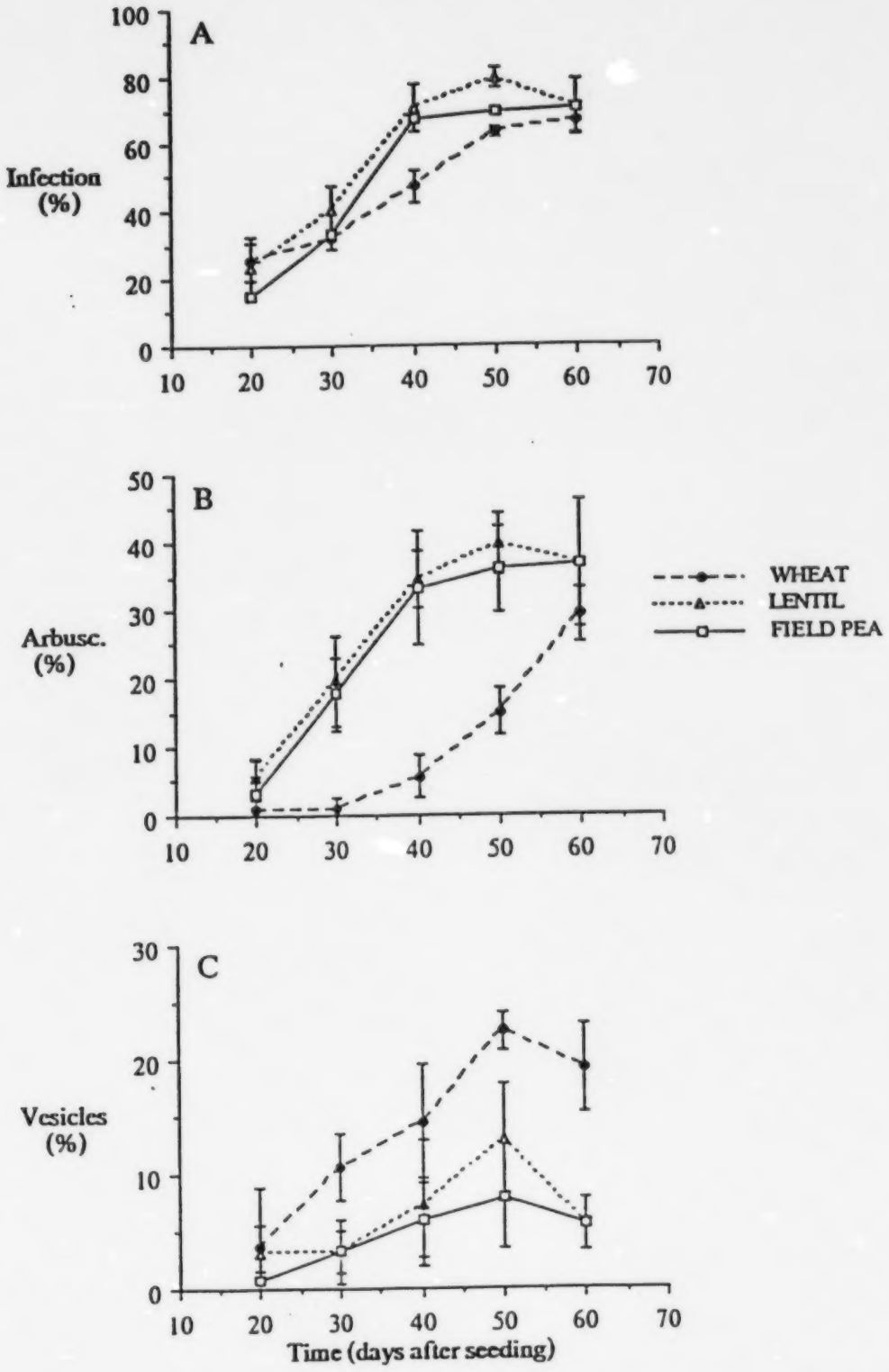

Figure 15. Development of VAM infection (A) and VAM structures including arbuscules (B) and vesicle (C) in wheat, lentil and field pea roots.

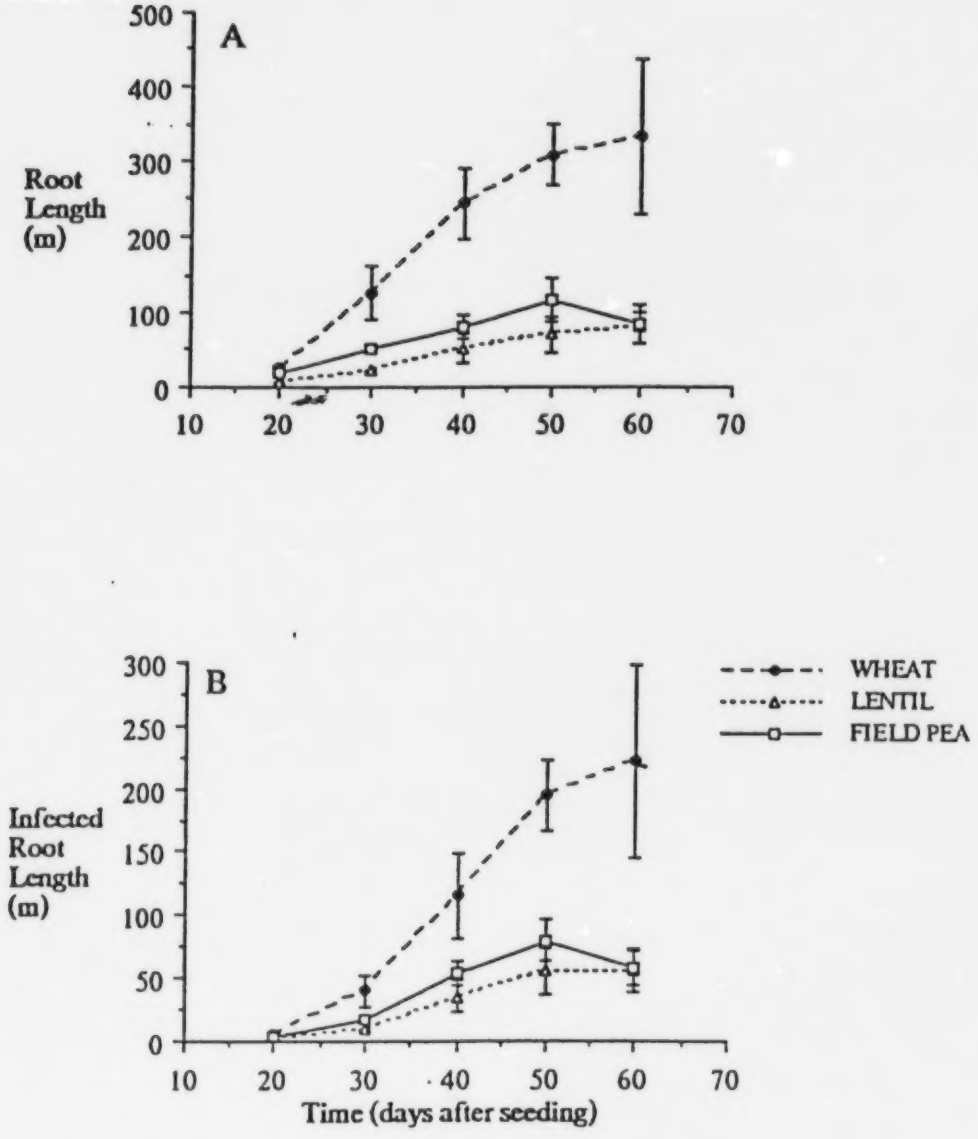

Figure 16. Root length (A) and infected root length (B), of wheat, lentil and field pea.

In addition to percent VAM infection, development of mycorrhizal structures including arbuscules and vesicles was also monitored (Fig. 15b, 15c). Arbuscules are complex 'bush-like' hyphal structures which play an important role in the exchange of metabolites between the VAM fungus and the host plant (White and Brown, 1979) Research has indicated that arbuscules contain high levels of phosphorus and calcium which are released to the host plant when the arbuscules break down (White and Brown, 1979)). Vesicles are globose bodies which form as a result of terminal swelling of VAM hyphae. These structures serve to store large quantities of fat which, on disintegration, are later transferred to cells (McLennan, 1962)

Lentil and field pea shared a similar pattern of arbuscule development; rapid increases in the relative numbers of arbuscules occurred between days 20 and 40 after which further development of arbuscules essentially ceased (Fig. 15b). In contrast, fewer arbuscules were observed in wheat roots between days 20 and 40, followed by a period of rapid increases in arbuscule development. At all but the first harvest, a higher percentage of vesicles was observed in wheat roots than in either lentil or field pea roots (Fig. 15c). Although all three crops shared a similar pattern of vesicle development, the rate of increase in the percentage of vesicles was greatest in wheat.

It is not known if the observed variations in VAM development are related to infection by different VAM species or if these variations reflect differences in the physiological development of the crop species. It is possible that the roots and root exudates of a particular crop preferentially encourage the development of one VAM species over another. Alternatively, physiological differences in plant development, which affect the carbohydrate and nutrient supply to plant symbionts, could stimulate differences in VAM development. In light of the important role that arbuscules and vesicles play in host crop nutrition, the observation that the development of these structures is host-crop dependant, as suggested by this study, is an important finding.

3.2.2 Growth Chamber Experiment #2 - The Effect of Phosphorus Application on the Development on VAM Infection in Wheat.

Results indicate that VAM infection was reduced by the application of phosphorus; the greatest reductions in VAM infection were associated with the highest level of phosphorus application (Fig.17a). Similarly, the presence of arbuscules was reduced by the application of increasing increments of phosphorus (Fig 17b). Arbuscules are complex 'bush-like' hyphal structures which play an important role in the exchange of metabolites between the VAM fungus and the host plant (Scannerini and Bonfante-Fasolo, 1983). Research has indicated that arbuscules contain high levels of phosphorus and calcium which are released to the host-plant when the arbuscules break down (White and Brown, 1979). In light of the important function that arbuscules play in phosphorus nutrition, it is particularly interesting that both the percentage of roots which were mycorrhizal and the percentage of roots containing arbuscules declined in response to increasing levels of fertilizer phosphorus levels. In light of the important role that arbuscules play in host crop nutrition, the observation that these structures are influenced by the application of phosphorus is an important finding. In contrast, the level of vesicles present in the roots appeared to be relatively unaffected by the level of phosphorus application (Figure 17c). Vesicles are globose bodies which form as a result of terminal swelling of VAM hyphae. These structures serve to store large quantities of fat which, on disintegration, are later transferred to cells (McLennan, 1966).

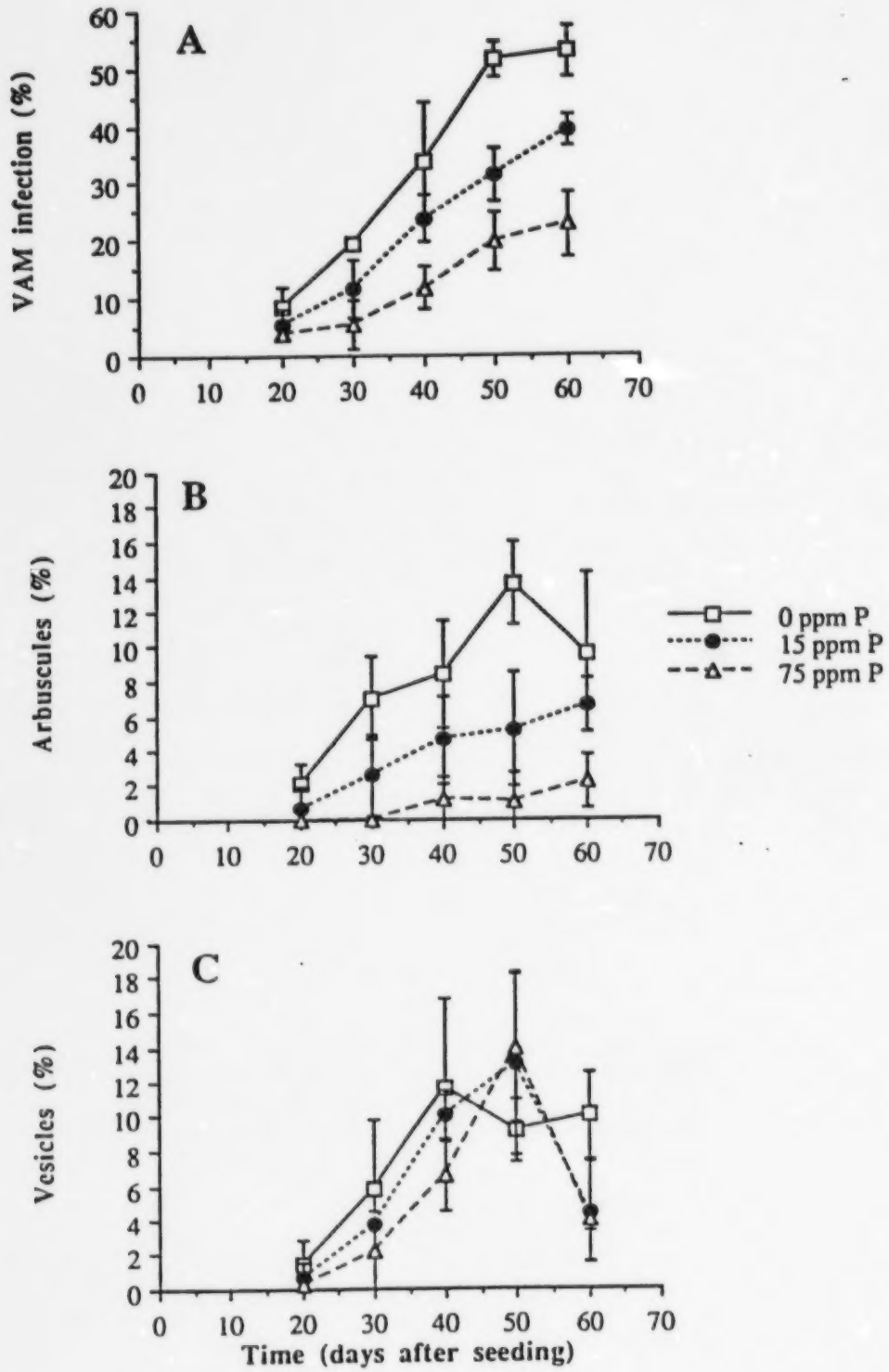

Figure 17. The effect of phosphorus application on percent VAM infection (A), arbuscules (B) and vesicles (C) in spring wheat.

3.2.3 Growth Chamber Experiment #3 - The Effect of Crop Rotation on the Development of VAM Infection in Wheat.

The effect of crop rotation on the percent VAM infection of wheat grown under growth chamber conditions is reported in Table 25. The corresponding ANOVA table is reported in Table 26. At each soil dilution, there was a significant reduction in the level of VAM infection of wheat grown in soils previously cropped to canola for two years. There was a significant effect on percent VAM infection due to soil dilution under each rotation which could be attributed to a reduction in VAM propagules. The decrease in percent VAM infection was highest in the barley - wheat (B-W) rotation soils under 0.5:1.5 soil :sand dilution. Because the decrease in nutrient concentration due to soil dilution was compensated for through the addition of nutrients, the differences in VAM infection can be attributed to differences in the inoculum level. Nutrient compensation was effective in maintaining crop growth in different soil dilutions at par with the non-diluted soils.

Table 25. Influence of crop rotation on percent VAM infection of wheat under growth chamber condition†

Soil dilutions	Crop Rotations		
	Canola-Canola	Canola-Barley	Barley-Wheat
0.5:1.5	28.5 (32.0)	32.5 (42.0)	33.8 (50.0)
1:1	35.5 (40.0)	43.5 (64.0)	58.5 (70.0)
2:0	32.0	41.5	61.0

† Average of four replications.
() Represents percent VAM infection without nutrient compensation.

Table 26. Sum of squares (SS) and mean sum of square (MSS) from analysis of variance for percent VAM infection of wheat.

Source	df	SS	MSS	F
Soil dilution	2	1518.50	759.25	
	10.43**			
Error *a*	9	654.91	72.76	
Crop Rotation	2	2230.16	1115.08	
	28.59**			
Crop Rotation X Soil dilution	4	669.33	167.33	4.29*
Error *b*	18		701.83	38.99
Total	35			

*, ** Indicate significance at P = 0.05 and 0.01, respectively.

These results indicate that the reduction in VAM infection associated with the inclusion of a non-host crop in a rotation is primarily due to the reduction in the levels of VAM spores (and infective propagules) in the soil.

5.0 Conclusions and Impact

Field studies conducted during 1988 and 1989 indicate that VAM are present in sufficient numbers in Saskatchewan soils to be an important factor in crop growth. At study sites located throughout Saskatchewan, it was observed that crops such as spring wheat, lentil and field pea were heavily infected with VAM. This observation suggests that VAM plays an important role in the nutrition and growth of these economically important crops in Saskatchewan.

In both field and growth chamber experiments, it was observed that the pattern of VAM infection appeared to be host specific, i.e., VAM infection of wheat roots did not follow the same pattern as that observed for lentil and field pea. Moreover, the level of VAM infection in wheat roots was generally less than that observed in the legume roots. A growth chamber experiment revealed, however, that although percent infection was generally less in wheat than in either of the legume crops, wheat root length exceeded the root length of both lentil and field pea. As a consequence, although percent VAM infection of wheat roots appears relatively low, the actual length of roots infected with VAM may indeed be relatively large. This is an important finding as it demonstrates that the relative importance of VAM infection in crop production and nutrition may not be related simply to the degree of infection; rather, the actual length of infected root may be a more representative indicator of the importance of VAM infection in any given crop.

The dominate species of VAM present in Saskatchewan soils, and the relative abundance of these species, differed from location to location. Eight different genera of VAM spores were isolated form Saskatchewan soils and identified as *Acaulospora* sp., *Gigaspora margarita*, *Glomus caledonium*, *G. fasciculatum*, *G. geosporum*, *G. mosseae*, *G. pallidum*, and *G. versiforme*. Growth chamber studies indicated that different VAM species differ in their ability to affect plant growth. These results suggest that the potential exists for the development of an effective VAM inoculant that could result in yield increases, particularly if applied to soils in which the indigenous VAM species is not an effective growth promoter. Isolation and identification of the different VAM species from Saskatchewan soils accomplished during the course of this research project represents an important step in the development of an effective VAM inoculant.

A number of factors were observed to play a role in the development of VAM infection in crop roots. For example, the extent of VAM infection was related to available soil phosphorus at seeding; generally high levels of available soil phosphorus resulted in a reduction in the levels of VAM infection in the crop roots. Similarly, application of fertilizer phosphorus subsequently reduced the levels of VAM infection. Thus, where phosphorus is limiting to crop growth, it is likely that VAM play a particularly important role in the procurement and uptake of this nutrient.

In both field and growth chamber studies, crop rotations were also observed to be an important factor in VAM infection. Although most crops grown in Saskatchewan are subject to VAM infection, canola is resistant. It was observed that when this resistant crop was grown in a crop rotation, the level of VAM infection in the crop grown on the canola stubble was generally reduced. It is likely that during the canola year, VAM spores continued to germinate in the soil but were unable to find a suitable host crop to infect. Alternatively, other soil microorganisms may have attacked and destroyed VAM spores. As a consequence, the spores began to die off during the canola year, leaving fewer spores to infect the subsequent crop. The inclusion of summerfallow in a rotation generally had a similar effect on the levels of VAM infection, i.e., crops grown on summerfallow exhibited reduced levels of VAM infection as compared to crops grown in a continuous rotation. In light of the adverse effect that non-host crops such as canola, and summerfallow have on

the population of VAM spores in the soil, VAM spore numbers could be strictly limited in rotations which included a year of summerfallow followed by canola, resulting in a reduction in the levels of VAM infection in the following crop. In a soil containing only marginal levels of available phosphorus, a reduction in the level of VAM infection could result in yield limitations or, alternatively, a greater need for phosphorus fertilization. Further research is required to determine the extent to which reduced spore numbers may be affecting the crop yields and phosphorus requirements.

Herbicide application was also investigated as a factor which may influence the levels of VAM infection in crops. Although results suggest that herbicide application may influence VAM infection, it is not known whether the herbicides directly affected the VAM, or if herbicide application altered crop growth, indirectly affecting the VAM. Alternatively, herbicide application may have influenced VAM infection through alteration of the weed spectrum associated with the different herbicides. Although many weeds are readily infected by VAM, some are resistant; thus, the presence of non-host weeds may have altered the subsequent production of VAM spores.

This study has significantly enhanced our knowledge regarding the occurrence and distribution of VAM in Saskatchewan soils. Widespread occurrence and heavy infection of wheat, lentil and field pea suggests that VAM play an important role in the nutrition and growth of these economically important crops. VAM population and infectivity are subject to the influence of a number of factors, including crop rotation. This indicates that there exists the potential to manipulate indigenous VAM through management practices in order to derive the greatest benefits to crop growth. Moreover, eight different VAM species have been isolated and identified which represents an important step towards the development of effective VAM inoculum.

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(D) Statement of Expenditures

UNIVERSITY OF SASKATCHEWAN
Agriculture Development Fund
"Occurrence and Significance of VA Mycorrhizal Symbiosis in Saskatchewan Field Crops"
Under the Direction of Dr. J.J. GERMIDA, SOIL SCIENCE
Statement of Receipts & Expenditures for the Period
February 1 - August 31, 1990

Receipts		
Overexpenditure as of January 31, 1990	$(13,282.00)	
Government of Saskatchewan - March, 1990	15,000.00	
Total funds available		$ 1,718.00
Expenditures		
Salaries	$7,964.00	
Fringe Benefits	572.96	
Materials & Supplies	697.20	
Travel	70.00	
Equipment	463.84	9,768.00
Overexpenditure as of August 31, 1990		$(8,050.00)

COPY FOR YOUR RECORDS

P.J. Germida
PROJECT DIRECTOR

M.G. SMITH
RESEARCH ACCOUNTANT

(E) Project Personnel

	Percentage of Time Involved in Project	Percentage of Salary from ADF Grant
Project Director:Dr. J.J. Germida	20%	-
Research Officer:F.L. Walley	100%	100%
Graduate Student :N.C. Talukdar	100%	Funded by Commonwealth Scholarship.
Research Assistant: S. Skinnidar (April 1989 - July 1990)	100%	100%
Research Assistant: B. Dahl (September 1989 - April 1990)*	100%	100%

* Part time (10 hours per week)

(F) Equipment purchases over $500.00 - None